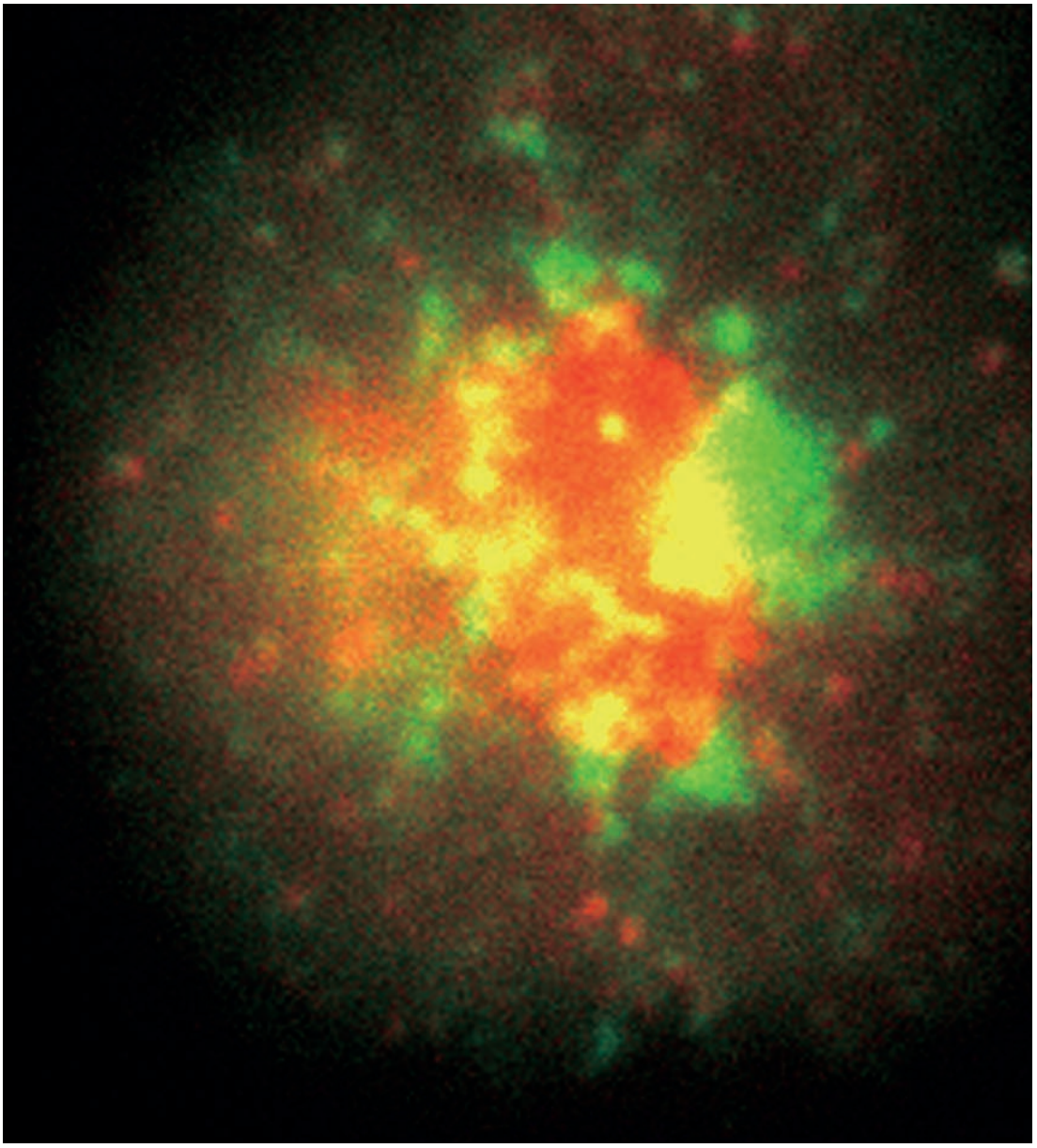

RIKEN IMS
Annual Report 2014

RIKEN Center for Integrative Medical Sciences

RIKEN Center for Integrative Medical Sciences Organization Chart

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Shigeo Koyasu

Deputy Director

Michiaki Kubo
Takashi Saito
Haruhiko Koseki
Toshihiro Tanaka

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- Lab. for Cell Signaling: **Takashi Saito**
- Lab. for Lymphocyte Differentiation: **Tomohiro Kurosaki**
- Lab. for Transcriptional Regulation: **Ichiro Taniuchi**
- Lab. for Immune Cell Systems: **Shigeo Koyasu**
- Lab. for Human Disease Models: **Fumihiko Ishikawa**
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- Lab. for Mucosal Immunity: **Sidonia Fagarasan**
- Lab. for Gut Homeostasis: **Kenya Honda**
- Lab. for Immune Homeostasis: **Shohei Hori**
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- Lab. for Metabolic Homeostasis: **Naoto Kubota**
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- Lab. for Inflammatory Regulation: **Takashi Tanaka**
- Lab. for Cytokine Regulation: **Masato Kubo**
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- Lab. for Integrated Bioinformatics: **Todd Duane Taylor**
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- Lab. for Integrated Cellular Systems: **Mariko Okada**
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- Lab. for Metabolomics: **Makoto Arita**

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- Lab. for Pharmacogenomics: **Taisei Mushiroda**
- Lab. for International Alliance on Genomic Research: **Ming Ta Michael Lee**
- Lab. for Cardiovascular Diseases: **Toshihiro Tanaka**
- Lab. for Autoimmune Diseases: **Kazuhiko Yamamoto**
- Lab. for Digestive Diseases: **Kazuaki Chayama**
- Lab. for Bone and Joint Diseases: **Shiro Ikegawa**
- Lab. for Endocrinology, Metabolism and Kidney Diseases: **Shiro Maeda**
- Lab. for Respiratory and Allergic Diseases: **Mayumi Tamari**

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- Lab. for Immunotherapy: **Shin-ichiro Fujii**
- Lab. for Vaccine Design: **Yasuyuki Ishii**
- Lab. for Allergic Disease: **Toshiaki Kawakami**
- RIKEN-TORII Joint Research Team: **Masaru Taniguchi**
- Drug Discovery Antibody Platform Unit: **Toshitada Takemori**

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Director's Report

RIKEN Center for Integrative Medical Sciences is a very new institute that began in 2013 as an ensemble of groups with diverse research backgrounds and interests. Because of this heterogeneity, several efforts have been undertaken to galvanize communication and collaboration among researchers within the Center, with the hope of developing exciting new research directions and even new research fields.

IMS held both its first and second Research Retreat in 2014. The aim of these two retreats was to give IMS scientists a better understanding of each other's research focuses, approaches and techniques. Nearly 200 IMS researchers and students spent two days together at each of the retreats. In the first one, held in Shonan, Kanagawa prefecture, each laboratory gave a general overview. For the second retreat, held in Narita, Chiba prefecture, the focus was on our many young researchers, who each had one minute to introduce their research focus. Those retreats provided excellent opportunities for PIs, young researchers and students to communicate with each other in a relaxed atmosphere.

This past year, IMS also had a series of Research Seminars every month. Because IMS researchers have different scientific backgrounds and cultures, speakers were requested to present their projects so that they could be understandable to researchers in other fields. It took more than a year for IMS researchers get to know each other and understand the focus and strategy of each laboratory. Now the Center is trying to enhance communication between young researchers and postdocs.

In addition to those formal events, I started a monthly "Happy Hour" where PIs can talk freely. Researchers tend to use different scientific languages and have different way of thinking based on their scientific fields, so I tried to motivate their free communication on any topic of their choosing. There are always difficulties when people from different cultures start something together, but I believe that when we finally overcome them, our Center's goal of "integrative medical sciences" will become a reality.

As evidence of progress toward this goal, several multidisciplinary center-wide research projects have already been launched to understand the pathogenesis of atopic dermatitis, type 2 diabetes, anaphylaxis, primary immunodeficiency and others, in which multiple research groups from different fields work interactively and synergistically to achieve their common objective - to understand the molecular and cellular networks that underlie homeostasis of each organ/tissue.

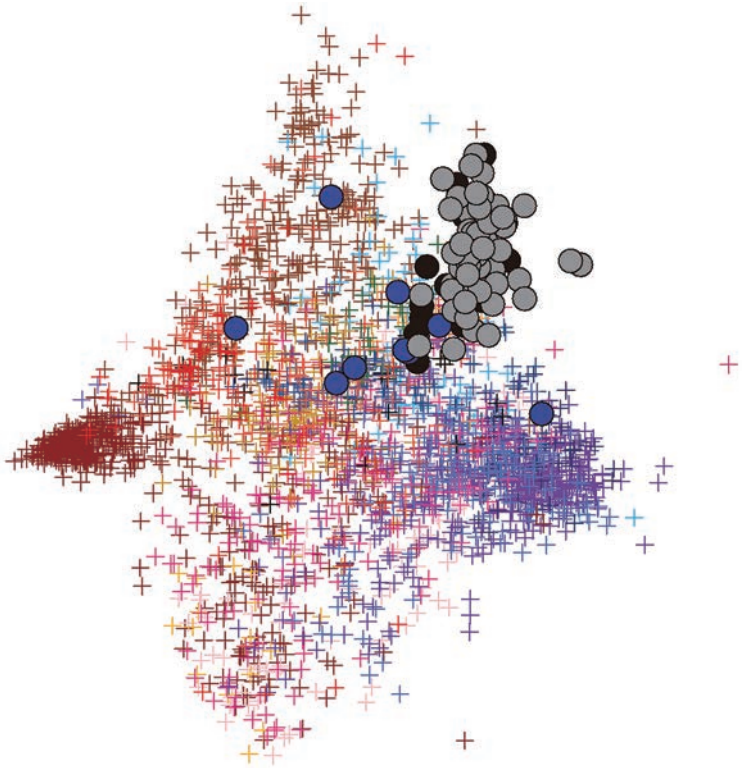
In the atopic dermatitis project, Drs. Yoshida and Masato Kubo are performing "wet lab" experimental studies, computational analyses and modeling are being done by Drs. Okada, Kitano and Ohara's labs, and Drs. Amagai, Tamari and Tsunoda are working to connect the findings between mouse and human atopic dermatitis. Meanwhile, Dr. Taylor's group has been developing an integrated database for the storage and distribution of the massive and various types of data generated by these different approaches.

In the type 2 diabetes project, a comprehensive multi-omics strategy is being used to study the role of host-gut microbiota interactions in the pathogenesis of this disease. Human samples are collected in collaboration with Drs. Kadowaki and Yamazaki (The Univ. of Tokyo), multi-omics analyses are done by Drs. Yamazaki, Hattori (The Univ. of Tokyo) and IMS labs led by Drs. Ohara, Ohno, Arita, Kubota, Maeda and Taylor. The goal is to identify disease risk factors by analyzing the meta data derived from this multi-omics approach.

In cancer genomics, IMS performed whole genome sequencing (WGS) and RNA-seq on 270 liver cancers and made comprehensive genomic profiles of liver cancers. IMS group led by Dr. Nakagawa has deposited WGS data of liver cancer and released them as part of the Japanese International Cancer Genome Consortium (ICGC) project. IMS is also a member of the Pan-Cancer Analysis of Whole Genomes (PCAWG) project, where data from ~3000 cancer WGS are analyzed in the same pipeline within the same computational environment by global efforts within ICGC/TCGA. IMS is arranging one of six academic "cloud" data centers worldwide and contributing to data analysis for driver genes, mutational signatures, immunogenomics and mitochondrial genomics of cancer in PCAWG, as well as providing data from 270 cancer WGS.

IMS continued to publish papers in significant journals in 2014. Dr. Mariko Okada reported a positive feedback mechanism within a kinse signaling complex that functions as a switch for NF-κB activation (*Science*, 2014). Dr. Kondo in Dr. Koseki's lab published a paper in *Cell* about polycomb regulatory mechanisms. Dr. Ikegawa discovered susceptibility genes for OPLL (ossification of the posterior longitudinal ligament of the spine), a disease in which there is ectopic calcification, particularly in the cervical spine (*Nature Genetics*, 2014). There were 226 papers published by IMS investigators in 2014, and I hope that in the coming year our publications will continue to flourish as pioneering works in integrative medical sciences.

Shigeo Koyasu
Director,
RIKEN Center for Integrative Medical Sciences



Part 1

Lab Activities

Core for Homeostatic Regulation

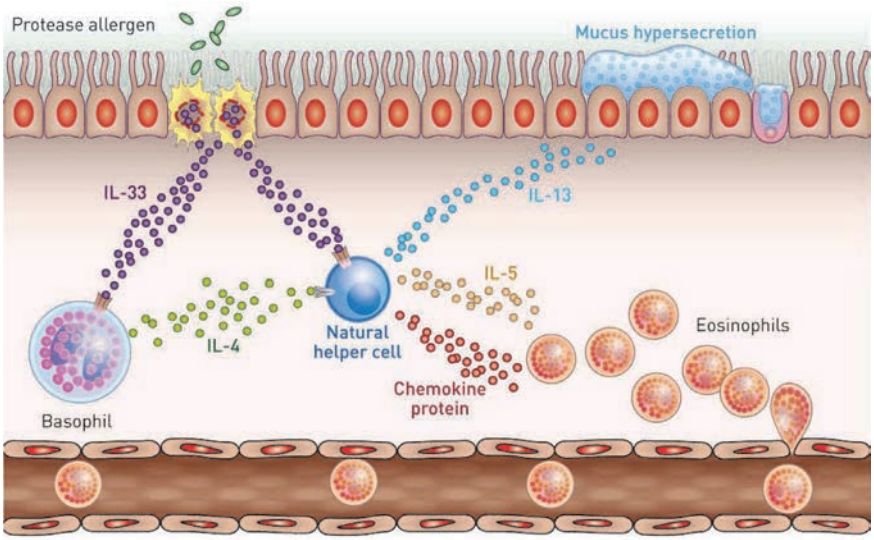


Figure: Lung inflammation causing asthma is induced by cross-talk between basophils and innate lymphoid cells ILC2 (natural helper cells): ILC2 produces IL-13 and IL-5 upon interaction with IL-4 derived from basophils.

The ultimate goal of the Core for Homeostatic Regulation is to elucidate the mechanisms of onset of human diseases and to create new scientific paradigms. This Core clarifies the regulation of homeostasis in individuals, focusing on their immune, metabolic and environmental response systems. In addition, the Core for Homeostatic Regulation will validate the disease models established by the Core for Precise Measuring and Modeling in a multitier timeframe from before to after the onset of diseases.

The Core for Homeostatic Regulation is composed of 15 laboratories, which are divided into four areas;

[1] Immune homeostasis

Cell signaling (T. Saito), Lymphocyte differentiation (T. Kurosaki), Immune homeostasis (S. Hori), Metabolic homeostasis (N. Kubota)

[2] Lymphocyte development

Transcriptional regulation (I. Taniuchi), Human disease models (F. Ishikawa)

[3] Mucosal immunity

Intestinal ecosystem (H. Ohno), Mucosal immunity (S. Fagarasan), Immune cell systems (S. Koyasu), Gut homeostasis (K. Honda), Immune crosstalk (H. Cheroutre)

[4] Allergy and inflammation

Skin homeostasis (M. Amagai), Inflammatory regulation (T. Tanaka), Cytokine regulation (M. Kubo)

All of these areas elucidate the basic mechanisms of immune regulation at cellular, tissue and systemic levels. We ultimately aim to analyze the onset of autoimmune diseases, metabolic disorders [1], primary immunodeficiency [2], inflammatory bowel disease and colitis [3], and atopic dermatitis and allergic diseases [4].

Laboratory for
Cell Signaling

Group Director: Takashi Saito

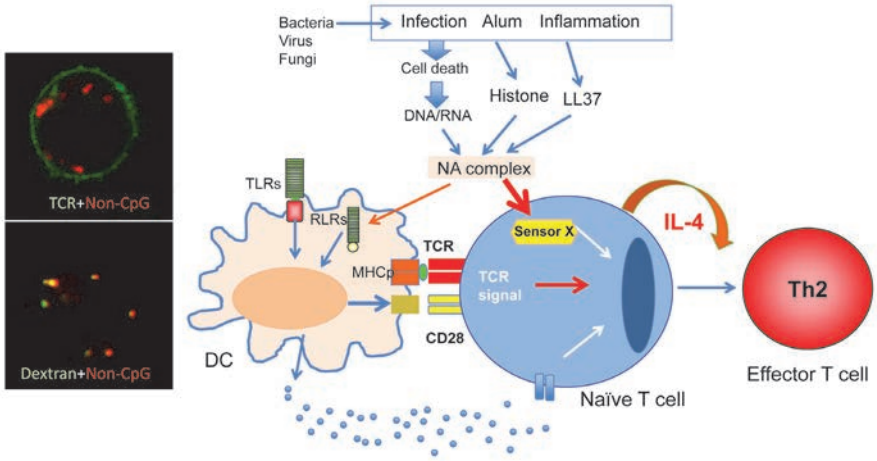


Figure: Sensing DNA/RNA from dying cells induces Th2 differentiation.
Nucleic acids are recognized by naïve T cells and induce their co-stimulation. T cells readily incorporate DNA into endosomes as indicated by colocalization with dextran (left) and promote growth and IL-4 production. DNA released from dying cells upon infection/inflammation induces the initial IL-4 production by naive T cells, which subsequently induces Th2 differentiation.

We have investigated the mechanism and regulation of activation, differentiation and function of T cells, particularly from the signaling perspective: spatiotemporal regulation of the assembly of signaling molecules, cytoskeletal regulation of activation, and co-regulation by innate signaling in T cells.

Functional analysis of innate-related signals for T cell activation revealed that effector Th1 but not Th2 cells were directly activated through TLR2 for IFNγ production. Nucleic acids (NA) induce T cell co-stimulation for cytokine production and proliferation, a process that is independent of TLR/RLRs. Unlike innate cells, any form of DNA can induce T cell co-stimulation, particularly when complexed with LL37 or histones. NA-mediated co-stimulation is induced by an unknown unique sensor and induces Th2 development. DNA released physiologically from dying cells upon infection or inflammation induces initial IL-4 production by naïve T cells and then their Th2 differentiation.

T cell activation is induced at the Immunological synapse (IS) formed between T cells and antigen-presenting cells. Analyzing the assembly of signaling molecules for T cell activation, we found that TCR microclusters as the initial site of T cell activation are surrounded by a ring of integrin/focal adhesion molecules that forms a structure termed a “microsynapse”. Microsynapses are formed transiently at the initial activation stage and are supported by the actin-cytoskeleton. They are essential for adhesion and activation of T cells, particularly upon weak TCR stimulation.

Recent Major Publications

Roncagalli R, Hauri S, Fiore F, Liang Y, Chen Z, Sansoni A, Kanduri K, Joly R, Malzac A, Lahdesmaki H, Lahesmaa R, Yamasaki S, Saito T, Malissen M, Aebersold R, Gstaiger M, Malissen B. Quantitative proteomic analysis of signalsome dynamics in primary T cells identifies the CD6 surface receptor as a LAT-independent TCR signaling hub. ***Nat Immunol*** 15, 384–92 (2014)

Kong KF, Fu G, Zhang Y, Yokosuka T, Casas J, Canonigo-Balancio AJ, Becart S, Kim G, Yates JR 3rd, Kronenberg M, Saito T, Gascoigne NR, Altman A. Protein kinase C-h controls CTLA-4-mediated regulatory T cell functions. ***Nat Immunol*** 15, 465–72 (2014)

Imanishi T, Ishihara C, Badr Mel S, Hashimoto-Tane A, Kimura Y, Kawai T, Takeuchi O, Ishii KJ, Taniguchi S, Noda T, Hirano H, Brombacher F, Barber GN, Akira S, Saito T. Nucleic acid sensing by T cells initiates Th2 cell differentiation. ***Nat Commun*** 5, 3566 (2014)

Invited presentation

Saito T. Regulation of initial T cell activation and functional differentiation. Novo Nordisk Innovation Summit. Tokyo, Japan. October, 2014

Saito T. T cell activation and differentiation upon direct sensing of nucleic acids. Cold Spring Harbor Asia Conferences. Suzhou, China. September, 2014.

Saito T. Imaging of lymphocyte activation. The 37th Naito Conference, Niseko, Japan. July, 2014.

Saito T. Direct sensing of nucleic acids by T cells induces Th2 differentiation. FASEB Science Research Conference. Snowmass, USA. June, 2014

Saito T. Nucleic acids sensing by T cells induces co-stimulation to initiate Th2 cell differentiation. EMBO Conference Lymphocyte signalling, Bertinoro, Italy. May, 2014

Laboratory for Lymphocyte Differentiation

Group Director: Tomohiro Kurosaki

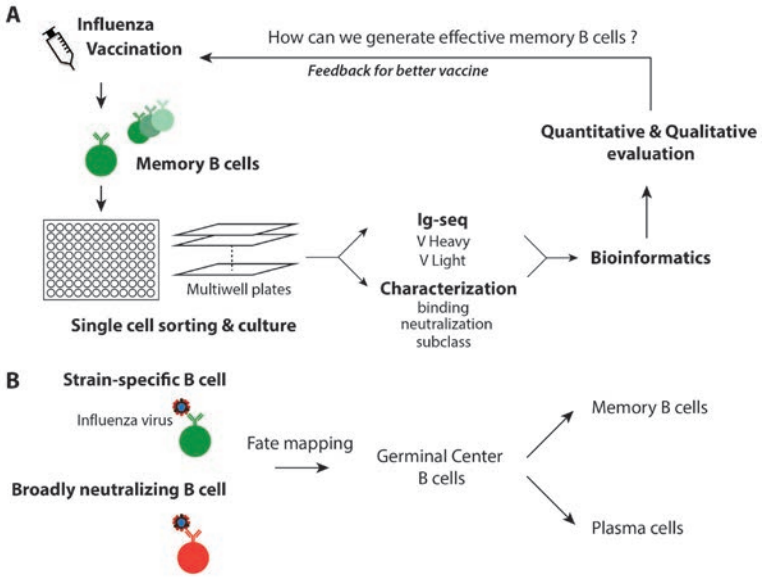


Figure: Tools for influenza vaccine study
A. Single cell culture system for quantitative and qualitative evaluation
B. Fate mapping of specific repertoires upon vaccination

Memory humoral responses are typically more rapid, have a greater magnitude, and consist of antibodies of higher affinity than in the primary response. Therefore, development of long-term B cell memory is an important goal of vaccination. Our laboratory has now focused on clarifying the mechanisms of how memory B cells are generated and how their unique functions are performed.

Mechanisms of memory B cell generation

In the case of $CD8^+$ T cells, transcription factors that regulate the terminal-effector versus memory T cell fates have been identified. Several of them are known to function in pairs that form counter-regulatory axes. Based on this idea, we hypothesized that similar mechanisms are operating to generate memory B cells. In this regard, we have focused upon the transcription factor Bach2, because it is known to be an antagonistic factor for the terminal-differentiation factor Blimp-1. After antigen-stimulation, the expression of Bach2 decreased in the expansion phase, and then increased in the contraction phase. By using conditional knockout mice, we found that Bach2 facilitated expansion of antigen-specific B cells and subsequent generation of memory B cells. Thus, restoration of Bach2 expression in the contraction phase is essential for memory B cell development.

Memory B cells against influenza virus

To develop better vaccines for various infectious diseases, we have developed a single B cell culture system to quantify the composition of the B cell repertoire and the specific activities against virus of individual components of the repertoire. For example, certain B cell repertoires may produce broadly cross-reactive antibodies that can protect against mutated influenza strains or different subtypes of the virus. Further, to trace virus-specific B cells, we have now generated B cell receptor knock-in mice to understand how these B cells acquire memory functions and survive longer. Overall, we have established several tools to understand memory B cell generation against influenza virus.

Recent Major Publications

Kurosaki T, Kometani K, Ise W. Memory B cells. ***Nat Rev Immunol*** (in press)

Ise W, Inoue T, McLachlan JB, Kometani K, Kubo M, Okada T, Kurosaki, T. Memory B cells contribute to rapid Bcl6 expression by memory TFH cells. ***Proc Natl Acad U S A*** 111, 11792–7 (2014)

Shinohara H, Behar M, Inoue K, Hiroshima M, Yasuda T, Nagashima T, Kimura S, Sanjo H, Maeda S, Yumoto N, Ki S, Akira S, Sako Y, *Hoffmann A, *Kurosaki T, *Okada-Hatakeyama M. Positive Feedback Within a Kinase Signaling Complex Functions as a Switch Mechanism for NF-κB Activation. (*co-corresponding authors) ***Science*** 344,760–4 (2014)

Invited presentations

Kurosaki T. Involvement of transcription factors in generation of memory B cells. The 43rd Annual Meeting of The Japanese Society for Immunology, Kyoto, Japan. December, 2014.

Kurosaki T. Mechanisms underlying rapid memory IgG responses. International Seminar Series: Institute for Basic Science (IBS), Gyeongbuk, Korea. December, 2014.

Kurosaki T. Regulatory functions of B lineage cells. France-Japan Immunology Meeting. Cassis, France. October, 2014.

Kurosaki T. Calcium signaling in B lymphocytes. 5th International Congress on Cell Membranes and Oxidative Stress: Focus on Calcium Signaling and TRP Channels, Isparta, Turkey. September, 2014.

Kurosaki T. Mechanisms underlying rapid memory IgG responses. The 2nd Symposium of International Immunological Memory and Vaccine Forum, La Jolla, USA. August, 2014.

Laboratory for Transcriptional Regulation

Group Director: Ichiro Taniuchi

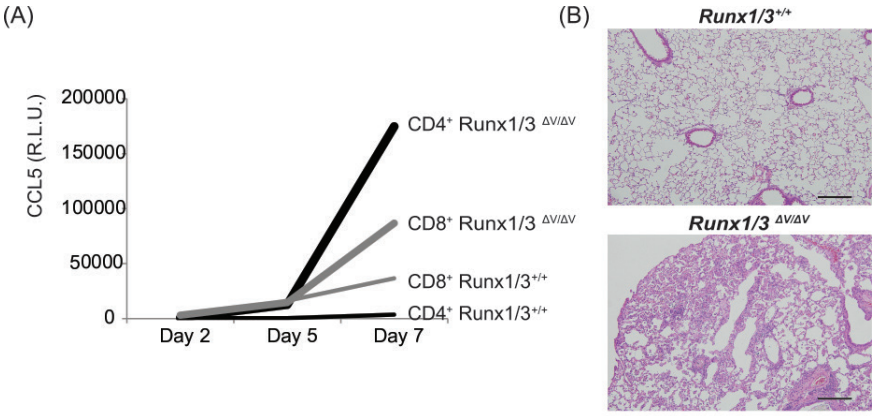


Figure: Derepression of CC chemokine expression by CD4⁺ T cells in Runx mutant mice.
Time course measurement of CCL5 secretion by CD4⁺ and CD8⁺ T cells of wild type (Runx1/3$^{+/+}$) and mutant mice lacking the VWRPY motif in both Runx1 and Runx3 proteins (Runx1/3$^{\Delta V/\Delta V}$) following *in vitro* activation (A) and lung histology of these mice (B). Scale bar: 20 μm.

Recent Major Publications

Majumder K, Koues OI, Chan EA, Kyle KE, Horowitz JE, Yang-Iott K, Bassing CH, Taniuchi I, Krangel MS, Oltz EM. Lineage-specific compaction of Tcrb requires a chromatin barrier to protect the function of a long-range tethering element. ***J Exp Med*** 212, 107–20 (2015)

Boucheron N, Tschismarov R, Göeschl L, Moser MA, Lagger S, Sakaguchi S, Winter M, Lenz F, Vitko D, Breitwieser FP, Müller L, Hassan H, Bennett KL, Colinge J, Schreiner W, Egawa T, Taniuchi I, Matthias P, Seiser C, Ellmeier W. CD4⁺ T cell lineage integrity is controlled by the histone deacetylases HDAC1 and HDAC2. ***Nat Immunol*** 15, 439–48 (2014)

Tanaka H, Naito T, Muroi S, Seo W, Chihara R, Miyamoto C, Kominami R, Taniuchi I. Epigenetic Thpok silencing limits the time window to choose CD4⁺ helper-lineage fate in the thymus. ***EMBO J*** 32, 1183–94 (2013)

Invited presentations

Taniuchi I. Transcriptional Regulation of T Cell Development in The Thymus. Functional Genomics and Experimental Medicine, Sendai, Japan. February, 2015.

Taniuchi I. Cell fate determination in the thymus. The 43rd Annual Meeting of The Japanese Society for Immunology, Kyoto, Japan. December, 2014.

Taniuchi I. Transcriptional Regulation of CD4/CD8 Lineage Choice. France-Japan Immunology Meeting, Cassis, France. October, 2014.

Taniuchi I. Transcriptional Control of Helper/Cytotoxic Lineage Choice in The Thymus. RIKEN IMS-JSI International Symposium on Immunology 2014, Yokohama, Japan. June, 2014.

Taniuchi I. Transcriptional Control of Helper/Cytotoxic Lineage Choice in The Thymus. Immunology 2014, AAI Annual Meeting, Pittsburgh, USA. May, 2014.

One of the major questions in developmental biology is how extracellular information is sensed by interface receptors and is integrated into a developmental program encoded by the genome. Research in my laboratory has been addressing this important issue by studying mechanisms that control helper versus cytotoxic T cell differentiation in the thymus as a useful and unique model system. We have previously proposed that an antagonistic interplay between two transcription factors, ThPOK and Runx, serves as a central mechanism to separate these cell fates. Thus, one of our research goals is to advance our understanding of how external information, recognition of peptide-MHC by the TCR in this case, is integrated into transcriptional and epigenetic control of the expression of these factors during the initial lineage selection process in the thymus. Currently we are focusing on functions of two nuclear factors, Bcl11b and SATB1, which both have turned out to be important for proper expression of *Thpok* and *Runx3* genes, in part by modifying local chromatin configuration.

Our second objective is to unravel functions of Runx transcription factor complexes, which act as heterodimers of Runx and the non-DNA binding Cbfb protein, in the control of hematopoietic cell differentiation. Our goal is to understand regulatory mechanisms that modulate the function of Runx complexes as well as to identify novel Runx target genes involved in immune cell development. We are addressing these questions mainly by analyzing a series of mutant mouse strains harboring specific mutations in the *Runx* family genes and by identification and characterization of Runx interacting molecules, including functional RNAs. Recently, we have found that the CC chemokine gene cluster is a novel Runx target, so that chemokine expression in CD4⁺ T cells should be repressed by Runx proteins to restrain the inflammatory response (Figure).

Laboratory for Immune Cell Systems

Group Director: Shigeo Koyasu

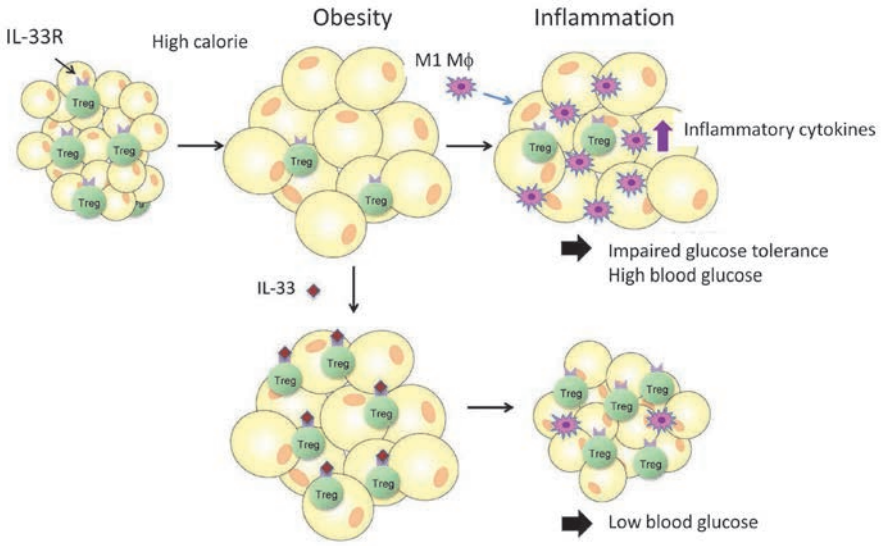


Figure: IL-33 regulates Tregs in visceral adipose tissue (VAT) to control adipose tissue homeostasis.
Tregs in VAT, but not lymphoid tissues, express receptors for IL-33. IL-33 maintains VAT Tregs and induces their proliferation. On a high fat diet, Treg numbers in VAT decreased, which was associated with elevated blood glucose levels and impaired glucose tolerance. Administration of IL-33 restored the VAT Treg numbers and improved blood glucose levels/glucose tolerance.

We focus on the fat-associated lymphoid cluster (FALC), a new lymphoid tissue in mouse, rat and human mesentery, and on the Natural Helper (NH) cell, an innate lymphocyte population, both of which we discovered. NH cells, now called group 2 innate lymphoid cells (ILC2), constitutively produce low levels of IL-5, IL-6 and IL-13 and support the proliferation of B1 cells in the peritoneal cavity and IgA production by B cells expressing surface IgA. NH cells respond to IL-33 and a combination of IL-2 and IL-25 during helminth infection and produce large amounts of IL-5 and IL-13. IL-5 induces eosinophilia and IL-13 induces goblet cell hyperplasia during the innate phase of helminth infection (Moro et al., Nature 463, 540-4, 2010). NH cells are involved in allergic inflammation in the lung and are involved in the steroid resistance of asthma. In collaboration with Dr. Masato Kubo's laboratory, we showed in 2014 that basophil-derived IL-4 in combination with IL-33 plays a pivotal role in the activation of NH cells in the lung inflammation triggered by a protease allergen, papain. We also examined the role of IL-33 in adipose tissue homeostasis in collaboration with Dr. Kallies' laboratory at WEHI, Australia. We found that regulatory T cells (Tregs) in visceral adipose tissue (VAT) uniformly express the IL-33 receptor and proliferate in response to IL-33. Generation and/or maintenance of VAT Tregs require IL-33, as IL-33-deficient mice lack VAT Treg, but they could be restored by administration of IL-33. Genetically obese NZO mice have reduced numbers of VAT Tregs and showed poor glucose tolerance along with high blood glucose. Administration of IL-33 ameliorated both Treg numbers and blood glucose levels/glucose tolerance. Our study demonstrated the importance of IL-33-mediated Treg induction/maintenance in adipose tissue homeostasis. We are studying the interaction between ILC2s and Tregs in VAT and the role of ILC2 in adipose tissue homeostasis.

Recent Major Publications

Vasanthakumar A, Moro K, Xin A, Liao Y, Gloury R, Kawamoto S, Fagarasan S, Mielke LA, Afshar-Sterle S, Masters SL, Nakae S, Saito H, Wentworth JM, Li P, Liao W, Leonard WJ, Smyth GK, Shi W, Nutt SL, Koyasu S, Kallies A. The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. ***Nat Immunol*** 16, 276–285 (2015)

Moro K, Koyasu S. Innate lymphoid cells, possible interaction with microbiota. ***Semin Immunopathol*** 37, 27–37 (2015)

Motomura Y, Morita H, Moro K, Nakae S, Artis D, Endo TA, Kuroki Y, Ohara O, Koyasu S, Kubo M. Basophil-derived interleukin-4 controls the function of natural helper cells, a member of ILC2s, in lung inflammation. ***Immunity*** 40, 758–71 (2014)

Invited Presentations

Moro K, Koyasu S. Innate Lymphoid cells and inflammation. The 43rd Annual Meeting of The Japanese Society for Immunology, Kyoto, Japan. December, 2014.

Koyasu S. Role of natural helper cells, a member of group 2 innate lymphoid cells (ILC2s), in allergic inflammation. 2014 NHRI/IBMS Joint International Conference on Inflammation & Disease, Taipei, Taiwan. October, 2014.

Koyasu S, Motomura Y, Moro K. The role of basophils in the activation of lung ILC2 in allergic inflammation in the lung. EMBO Conference on Innate Lymphoid Cells 2014, Paris, France. October, 2014.

Koyasu S, Moro K. Role of Natural Helper Cells, a member of group 2 innate lymphoid cells (ILC2s), in health and diseases. Cold Spring Harbor Asia Conferences: Frontiers of Immunology in Health and Diseases, Suzhou, China. September, 2014.

Laboratory for Human Disease Models

Group Director: Fumihiko Ishikawa

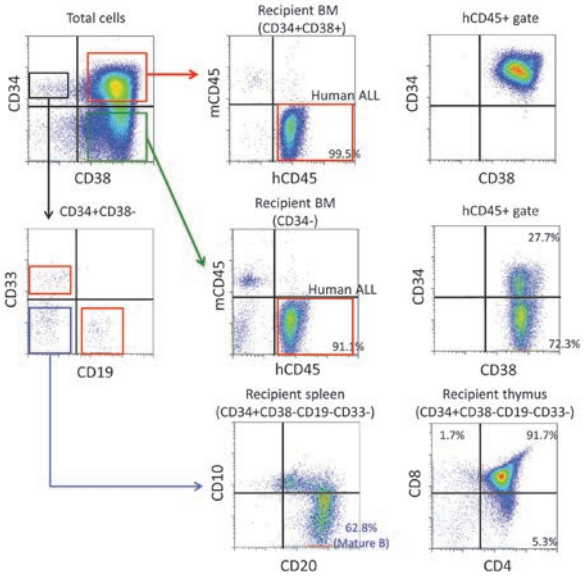


Figure: CD34⁺CD38⁺ and CD34⁻ cells, but not CD34⁺CD38⁻ cells, initiate leukemia in vivo.
CD34⁺CD38⁺, CD34⁻CD38⁺, and CD34⁺CD38⁻CD19⁻CD33⁻ cells were purified from human leukemic bone marrow samples and transplanted into newborn NOD/SCID/Il2rgKO mice. Recipients of CD34⁺CD38⁺ and CD34⁻ cells developed leukemia. CD34⁻ leukemic cells generated CD34⁺CD38⁺ cells in the recipient organs. CD34⁺CD38⁻CD19⁻CD33⁻ cells were evaluated as normal HSC/HPC based on their capacity to give rise to normal B and T cell lineages.

To directly analyze human hematopoiesis and immunity *in vivo*, we previously established a newborn NOD/SCID/Il2rgKO (NSG) xenotransplant model enabling us to achieve high levels of human hematopoietic chimerism in the recipient mouse organs. Based on this model, we are creating mice with a humanized environment to better support human lympho/hematopoiesis, such as HLA-expressing humanized mice (Proc Natl Acad Sci USA, 2010) or human stem cell factor (SCF)-expressing humanized mice (Blood, 2012).

During FY2014 we have developed a humanized mouse model for a leukemia associated with rearrangement of the mixed lineage leukemia (*MLL*) gene. In pediatric hematology/oncology, many children with acute leukemia can be cured with chemotherapy and/or stem cell transplantation. Nevertheless, a particular leukemia with the *MLL*-rearrangement is still difficult to treat, and more than half of the patients with MLL leukemia will succumb to the disease. This rearrangement involves translocation of the *MLL* gene on chromosome 11q23 with other chromosomes, generating fusion genes with multiple partners such as *AF4*, *AF9*, and *ENL* and leading to the initiation leukemia.

Considering the poor prognosis of MLL leukemia, our laboratory started a collaboration with Prof. Mizutani and his colleagues at JPLSG (Japan Pediatric Leukemia Study Group) in 2010 to collect patient samples nationwide. After we obtained the samples, we sought to identify leukemia-initiating cells (LIC) by injecting different cellular fractions, CD34⁺CD38⁻, CD34⁺CD38⁺, and CD34⁻ cells into NOD/SCID/Il2rgKO newborns. We successfully recapitulated patient leukemia status in the recipient mice and identified LICs in each of the different translocations involving the *MLL* gene. Simultaneously, we found that CD34⁺CD38⁻CD19⁻CD33⁻ cells are enriched for normal HSC/HPC activity. Through a comparison of gene expression profiles between normal HSCs and LICs we found that, among plasma membrane molecules, CD9, CD32, CD24, and CD180 were expressed by patient LICs but not by their HSCs. We will further validate whether these cell surface molecules are potential therapeutic targets for MLL-leukemia.

Recent Major Publications

Aoki Y, Watanabe T, Saito Y, Kuroki Y, Hijikata A, Takagi M, Tomizawa D, Eguchi M, Eguchi-Ishimae M, Kaneko A, Ono R, Sato K, Suzuki N, Fujiki S, Koh K, Ishii E, Shultz LD, Ohara O, Mizutani S, Ishikawa F. Identification of CD34⁺ and CD34⁻ leukemia-initiating cells in MLL-rearranged human acute lymphoblastic leukemia. ***Blood*** 125, 967–80 (2015)

Saito Y, Yuki H, Kuratani M, Hashizume Y, Takagi S, Honma T, Tanaka A, Shirouzu M, Mikuni J, Handa N, Ogahara I, Sone A, Najima Y, Tomabechi Y, Wakiyama M, Uchida N, Tomizawa-Murasawa M, Kaneko A, Tanaka S, Suzuki N, Kajita H, Aoki Y, Ohara O, Shultz LD, Fukami T, Gogo T, Taniguchi S, Yokoyama S, Ishikawa F. A pyrrolo-pyrimidine derivative targets human primary AML stem cells in vivo. ***Sci Transl Med*** 5, 181ra52 (2013)

Invited Presentations

Ishikawa F. Humanized mouse research. Human Immunology Forum, Kyoto, Japan. December, 2014.

Ishikawa F. Developing therapeutic strategies human AML stem cells. The 73rd Annual Meeting of the Japanese Cancer Association, Yokohama, Japan. September, 2014.

Ishikawa F. Humanized mouse for understanding normal and diseased human immunity. The 42nd Annual Meeting of The Japan Society for Clinical Immunology, Tokyo, Japan. September, 2014.

Ishikawa F. Understanding human immune system and diseases using humanized mouse system. The 18th Annual Meeting of Japanese Association of Cancer Immunology, Matsuyama, Japan. July, 2014.

Ishikawa F. Developing Therapeutic Strategies Targeting Human AML Stem Cells. The 5th JSH International Symposium 2014 in Hamamatsu, Hamamatsu, Japan. May, 2014.

Laboratory for Intestinal Ecosystem

Group Director: Hiroshi Ohno

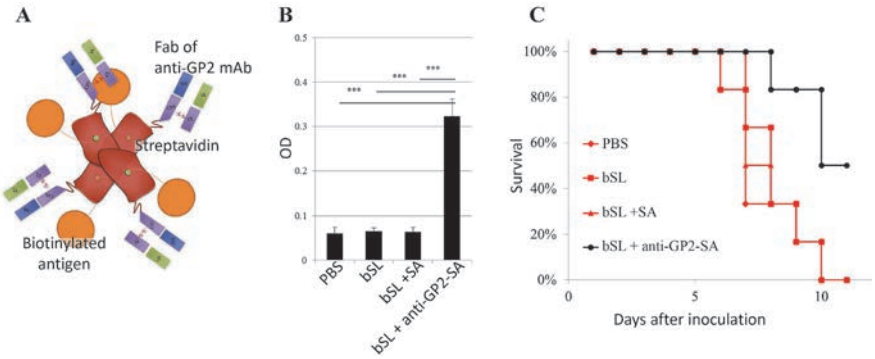


Figure: Chimeric molecule composed of a GP2 monoclonal antibody with streptavidin can efficiently deliver biotinylated *Salmonella* antigens to the mucosal immune system for *Salmonella*-specific fecal IgA secretion and induction of protective immunity against *Salmonella* infection.

A, Schematic representation of the chimeric molecule composed of the Fab from an anti-GP2 monoclonal antibody with streptavidin (anti-GP2-SA). B, Mice were orally administered with PBS, biotinylated *Salmonella* Typhimurium lysate (bSL), bSL conjugated with streptavidin (SA), or bSL conjugated with anti-GP2-SA three times, and fecal S. Typhimurium-specific IgA was measured. ***P < 0.001. C, Mice immunized in (B) were orally infected with *S.* Typhimurium and their survival was observed. (Refer to Shima et al., Int Immunol 26, 619–25, 2014 for details)

Recent Major Publications

Obata Y, Kimura, S, Nakato G, Iizuka K, Miyagawa Y, Nakamura Y, Furusawa Y, Sugiyama M, Suzuki K, Ebisawa M, Fujimura Y, Yoshida H, Iwanaga T, Hase K, Ohno H. Epithelial-stromal interaction via Notch signaling is essential for the full maturation of gut-associated lymphoid tissues in mice. ***EMBO Rep*** 15, 1297–304 (2014)

Shima H, Watanabe T, Fukuda S, Fukuoka S, Ohara O, Ohno H. A novel mucosal vaccine targeting Peyer's patch M cells induces protective antigen-specific IgA responses. ***Int Imunol*** 26, 619–25 (2014)

Kato T, Fukuda S, Fujiwara A, Suda W, Hattori M, Kikuchi J, Ohno, H. Multiple omics uncovers host-gut microbial mutualism during prebiotic fructooligosaccharide supplementation. ***DNA Res*** 21, 469–80 (2014)

Invited Presentations

Ohno H. Gut ecosystem, diseases and host defense. The 35th Annual Meeting of Japan Society for the Study of Obesity, Miyazaki Japan. October, 2014.

Ohno H. Gut microbe-derived butyrate can induce colonic Treg differentiation. 2014 Cold Spring Harbor Asia Conferences-Evolutionary Genetics and Genomics, Suzhou China. October, 2014.

Ohno H. Commensal microbe-derived butyrate epigenetically induces colonic regulatory T cell differentiation. International Conference of Beneficial Microbes 2014, Penang, Malaysia. May, 2014.

Ohno H. The Role of Short-Chain Fatty Acid Produced by Gut Microbiome on the Host Defense Mechanisms. Digestive Disease Week 2014, Chicago, USA. May, 2014.

Ohno H. Gut microbiota and metabolic regulation. The 87th Annual Meeting of the Japan Endocrine Society, Fukuoka, Japan. April, 2014.

Gut microbiota play important roles in normal physiology as well as pathology of the host. However, the gut does not unconditionally accept commensal microorganisms. Our intestinal immune system somehow senses the type and quantity of bacteria existing in the gut lumen and tries to contain the total number and composition of the gut microbiome. The aim of this laboratory is to understand the mechanisms by which the host and its gut commensal microbiota interact, especially focusing on how gut microbes are delivered across the intestinal epithelial barrier to be recognized by the intestinal immune system, how gut microbiota shape host defense and immune systems, and how host-gut microbiota interactions affect host health and disease status.

The delivery of particulate antigens such as bacteria is thought to be mainly achieved by a unique epithelial cell subset, M cells, residing in a limited region of the epithelial layer covering the lymphoid follicles of gut-associated lymphoid tissue such as Peyer's patches. We are studying the function and differentiation of M cells at the molecular mechanistic level. We have identified several bacterial uptake receptors on M cells, such as glycoprotein 2 (GP2) and cellular prion protein. GP2 could be a good target for antigen delivery for efficient vaccination. With this in mind, we screened GP2-specific aptamers. We also showed that a chimeric molecule consisting of a GP2 monoclonal antibody and streptavidin was able to efficiently deliver biotinylated antigens to PPs for the induction of antigen-specific protective IgA.

For study of host-gut microbiota interactions, we employ a comprehensive multiple omics approach, combining exhaustive metagenomic, (meta)transcriptomic, and metabolomic analyses. By applying this approach, we have identified gut microbes and their metabolites possibly involved in the rapid IgA secretion that occurs upon administration of the prebiotic fructooligosaccharide.

Laboratory for Mucosal Immunity

Team Leader: Sidonia Fagarasan

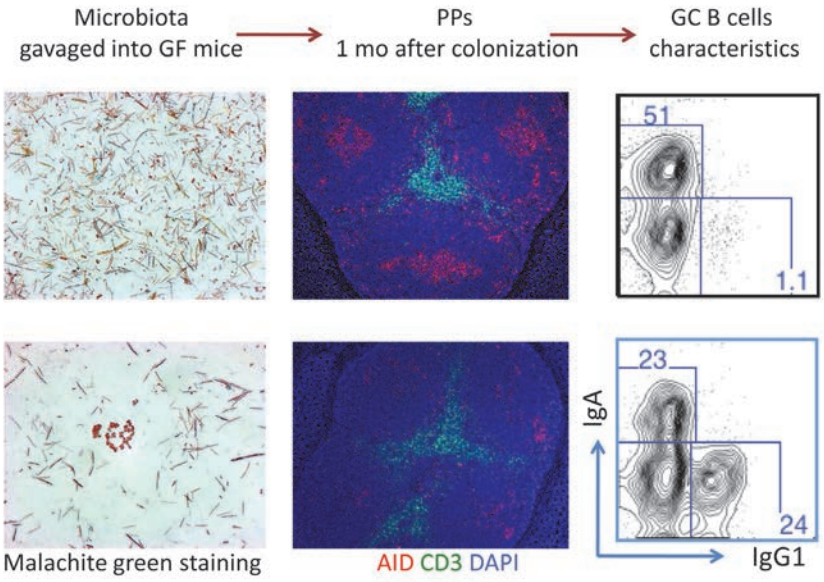


Figure: Maturation of gut immune responses and IgA-inducing properties in germ-free mice gavaged with complex microbial communities regulated by Foxp3⁺ T cells.
The upper panels show a complex microbiota from a T cell-deficient mouse transferred with Foxp3⁺ T cells, and its effect when transplanted into germ-free mice. The lower panels show a low diversity microbiota from a T cell-deficient mouse transferred with naïve CD4⁺T cells. Germinal center induction and preferential switching to IgA by GC B cells in Peyer's patches (PP) was observed in GF mice colonized with the complex microbiota (typical mucosal response). Note the poor inductive capacity and elicitation of IgG responses by low diversity microbiota [mixed mucosal and systemic (inflammatory) response].

Immunoglobulin A (IgA) is the major effector molecule of adaptive immunity in the gut, and its absence severely affects the balance of gut bacterial communities, resulting in massive activation of the immune system throughout the entire body.

Furthermore, fine-tuning of IgA selection in the germinal centers (GC) of Peyer's patches, which depends on Foxp3⁺ T cells acting as follicular regulatory T cells (TFR cells), also impacts on the gut microbiota. Interestingly, the regulation of IgAs by TFR is required for the maintenance in the gut of species belonging particularly to Firmicutes, which are spore-forming bacteria that are major contributors to the richness of the microbiota (Kawamoto et al., Immunity, 2014). Thus in the absence of Foxp3⁺ T cells, we observed impoverished bacterial communities in the gut characterized by uncontrolled expansion of a few bacterial species belonging to Bacteroidetes.

These observations raise the question as to whether different types of bacterial communities are "seen" differently by the immune system and, if so, whether they trigger distinct types of immune responses. We found that a complex and balanced microbiota promptly elicited immune responses with typical mucosal characteristics, namely induction of GCs with IgA supporting properties and induction or expansion of CD4⁺ T cells, especially of Foxp3⁺ T cells. In contrast, a simple and skewed microbiota provoked responses with mixed mucosal and systemic characteristics (i.e., GC B cells switching not only to IgA but also to IgG1, and possibly to IgE). Our data suggest that the immune system recognizes complex and balanced microbial communities as having a "gut signature" and responds by adaptations that foster the maintenance of such complex bacterial structures.

Recent Major Publications

Kumar R, Bach MP, Mainoldi F, Maruya M, Kishigami S, Jumaa H, Wakayama T, Kanagawa O, Fagarasan S, Casola S. Antibody repertoire diversification through VH gene replacement in mice cloned from an IgA plasma cell. ***Proc Natl Acad Sci U S A*** 112, E450–7 (2015)

Kawamoto S, Maruya M, Kato LM, Suda W, Atarashi K, Doi Y, Tsutsui Y, Qin H, Honda K, Okada T, Hattori M, Fagarasan S. Foxp3⁺ T cells regulate immunoglobulin A selection and facilitate diversification of bacterial species responsible for immune homeostasis. ***Immunity*** 41, 152–65 (2014)

Magri G, Miyajima M, Bascones S, Mortha A, Puga I, Cassis L, Barra CM, Comerma L, Chudnovskiy A, Gentile M, Llige D, Cols M, Serrano S, Arostegui JI, Juan M, Yague J, Merad M, Fagarasan S, Cerutti A. Innate lymphoid cells integrate stromal and immunological signals to enhance antibody production by splenic marginal zone B cells. ***Nat Immunol*** 15, 354–64 (2014)

Invited presentations

Fagarasan S. Regulation of gut microbiota by Foxp3 and IgA. Herrenhausen Conference: "Beyond the Intestinal Microbiome – From Signatures to Therapy", Hanover, Germany. October, 2014.

Fagarasan S. Regulation of gut microbiota by Foxp3 and IgA. 18th Germinal Centre Conference, Uddevalla, Sweden. September, 2014.

Fagarasan S. Symbiotic regulatory loop between Foxp3⁺ T cells, IgA and gut microbiota. The 2014 Annual Meeting of Korean Society for Biochemistry and Molecular Biology, Seoul, Korea. May, 2014.

Fagarasan S. Symbiotic regulatory loop between Foxp3⁺ T cells, IgA and microbiota in the gut. 109th International Titisee Conference: Microbiome-host mutualism in the shaping of host immunity, Titisee, Germany. April, 2014.

Laboratory for Gut Homeostasis

Team Leader: Kenya Honda

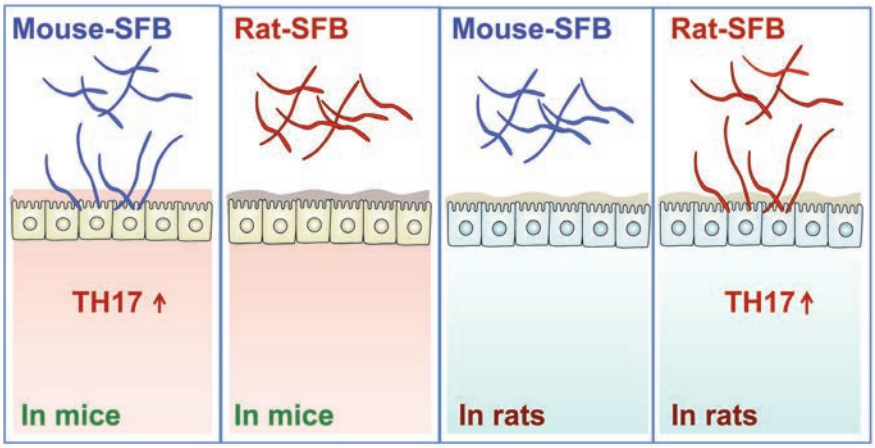


Figure: Schematic showing host-specific epithelial adhesion and Th17 cell induction by segmented filamentous bacteria (SFB).
SFB induce Th17 cells in the small intestine of its normal host, but not in a non-physiological host. Adhesion to epithelial cells is always associated with an increase of Th17 cells. Therefore, activation of epithelial cells by bacterial adhesion may be a prerequisite for SFB-mediated induction of Th17 cells.

The intestinal mucosa has a unique immune system composed of a variety of immune cell populations. The development and function of these gut-unique cells are known to be affected by the presence of the gut microbiota. Last year, we showed that a subset of gut commensal microbes belonging to the class *Clostridia* was responsible for triggering production of colonic $CD4^+Foxp3^+$ regulatory T (Treg) cells, a key therapeutic target in a number of autoimmune and inflammatory diseases. This year, we have zeroed in on another subset of $CD4^+$ T cells, Th17 cells. Intestinal Th17 cells are induced and accumulate in response to colonization with a subgroup of intestinal microbes, such as segmented filamentous bacteria (SFB) and several extracellular pathogens. However, it was not clear what elements of these microbes specifically elicited Th17 versus other immune cell responses in the intestine. We hypothesized that adhesion of microbes to intestinal epithelial cells (ECs) was one of the critical cues for Th17 induction. SFB indigenous to mice (M-SFB) and rats (R-SFB) are genetically distinct host-specific members of the gut microbiota. Upon monocolonization in germ-free mice or rats, M-SFB and R-SFB showed host-specific adhesion to ECs of the small intestine (SI), accompanied by host-specific induction of Th17 cells. Adherent SFB elicited a unique gene-expression program in SI ECs and an SFB antigen-specific Th17 response. Upon monocolonization in mice, intestinal pathogens including *Citrobacter rodentium* and *Escherichia coli* O157:H7 triggered similar Th17 responses, whereas their adhesion-defective mutants failed to do so. Moreover, a mixture of 20 bacterial strains, which were selected and isolated from fecal samples from a patient with ulcerative colitis (UC) on the basis of the ability to cause a robust induction of Th17 cells in the mouse colon, also exhibited EC-adhesive characteristics. These findings define Th17 induction as a host response to EC-adhesive commensal and pathogenic microbes and provide clues into the better development of mucosal vaccines (manuscript submitted).

Recent Major Publications

Kawamoto S, Maruya M, Kato LM, Suda W, Atarashi K, Doi Y, Tsutsui Y, Qin H, Honda K, Okada T, Hattori M, Fagarasan S. Foxp3$^+$ T cells regulate immunoglobulin a selection and facilitate diversification of bacterial species responsible for immune homeostasis. ***Immunity*** 41, 152–65 (2014)

Narushima S, Sugiura Y, Oshima K, Atarashi K, Hattori M, Suematsu M, Honda K*. Characterization of the 17 strains of regulatory T cell-inducing human-derived Clostridia. ***Gut Microbes*** 5, 333–9 (2014).

Obata Y, Furusawa Y, Endo TA, Sharif J, Takahashi D, Atarashi K, Nakayama M, Onawa S, Fujimura Y, Takahashi M, Ikawa T, Otsubo T, Kawamura YI, Dohi T, Tajima S, Masumoto H, Ohara O, Honda K, Hori S, Ohno H, Koseki H, Hase K. The epigenetic regulator Uhrf1 facilitates the proliferation and maturation of colonic regulatory T cells. ***Nat Immunol*** 15, 571–9 (2014)

Invited Presentations

Honda K. Th17 responses to epithelial adhesive intestinal microbes. Cold Spring Harbor Asia Conferences, Suzhou, China. September, 2014.

Honda K. Regulation of Th17 and Treg cells by the gut microbiota. 109th International Titisee Conference Microbiome-host mutualism in the shaping of host immunity, Titisee, Germany. April, 2014.

Laboratory for Immune Homeostasis

Team Leader: Shohei Hori

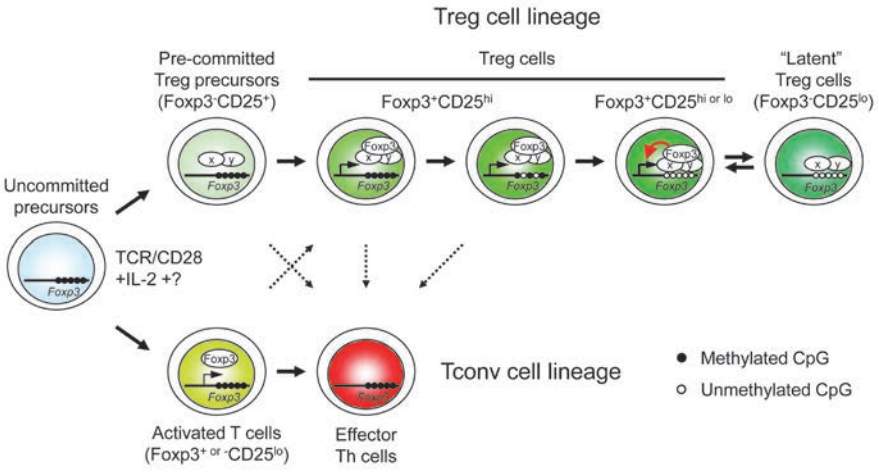


Figure: A model of regulatory T cell fate determination and maintenance
During Treg cell differentiation, uncommitted precursor cells adopt either Treg or conventional T (Tconv) cell fates. The commitment to the Treg cell fate is made before (and thus independently of) Foxp3 expression and is executed by a transcription factor network elicited by extrinsic signals from the extracellular environment. The same signals also induce epigenetic changes, including DNA demethylation of the *Foxp3* locus. Foxp3 is incorporated into the pre-existing transcription factor network and the resulting "Foxp3 interactome" establishes the characteristic Treg cell phenotype and function in cooperation with the epigenetic modifications. Although Treg cells may down-regulate Foxp3 expression under certain circumstances, these "latent" Treg cells retain the epigenetic memory of, and thus remain committed to, the Treg cell fate. On the other hand, when expressed in activated T cells without engagement of the epigenetic changes and the Foxp3 interactome, Foxp3 by itself cannot establish the characteristic Treg cell phenotype.

Regulatory T (Treg) cells expressing the transcription factor Foxp3 play an indispensable role in the establishment and maintenance of immunological self-tolerance and tissue homeostasis. This concept was firmly established by the finding that defective generation or function of Treg cells underlies a fatal autoimmune disease that develops in Foxp3-mutant mice and in humans suffering from the IPEX syndrome. Recent findings that Foxp3$^+$ Treg cells exert tissue-protective or immune-suppressive functions under diverse circumstances have raised the question of what mechanisms ensure the robustness of Treg cell functions, and thus of immunological self-tolerance, in the face of various unpredictable perturbations in the extracellular environment. To answer this question, we have focused on the mechanisms that control lineage stability and adaptability of Treg cells in changing environments.

We have previously shown that Foxp3 expression *per se* does not specify the Treg cell lineage in that activated conventional T cells can promiscuously and transiently express Foxp3 while committed Treg cells can transiently and reversibly down-regulate Foxp3. Despite this phenotypic plasticity, Treg cells retain epigenetic memory of, and thus remain committed to, Foxp3 expression and regulatory functions. We are now addressing the mechanisms underlying this epigenetic memory of Treg cell phenotype and function.

Another focus of our research is to understand how Foxp3 and Treg cells control immunological self-tolerance and tissue homeostasis. To address this question, we have addressed how Foxp3 gene mutations found in human IPEX impinge on Treg cells *in vivo* using knock-in mutagenesis in mice. Our analysis revealed that, while many mutations are amorphic or hypomorphic, one particular mutation acts as a gain-of-function mutation with respect to DNA binding and preferentially impairs Treg cell homeostasis in non-lymphoid tissues. By taking advantage of this unique animal model, we are currently investigating how Treg cells adapt to diverse and fluctuating tissue environments.

Recent Major Publications

Hori S. Lineage stability and phenotypic plasticity of Foxp3$^+$ regulatory T cells. ***Immunol Rev*** 259, 159–72 (2014)

Obata Y, Furusawa Y, Endo TA, Sharif J, Takahashi D, Atarashi K, Nakayama M, Onawa S, Fujimura Y, Takahashi M, Ikawa T, Otsubo T, Kawamura YI, Dohi T, Tajima S, Masumoto H, Ohara O, Honda K, Hori S, Ohno H, Koseki H, Hase K. The epigenetic regulator Uhrf1 facilitates the proliferation and maturation of colonic regulatory T cells. ***Nat Immunol*** 15: 571–9 (2014)

Miyao T, Floess S, Setoguchi R, Luche H, Fehling HJ, Waldmann H, Huehn J, Hori S. Plasticity of Foxp3$^+$ T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. ***Immunity*** 36, 262–75 (2012)

Invited presentations

Hori S. Foxp3-dependent control of regulatory T cell function and homeostasis. Academy of Immunology and Microbiology seminar series, Pohang, the Republic of Korea, March, 2015.

Hori S. Understanding genotype-phenotype relationships: lessons learned from disease-causing Foxp3 mutations. Monash Univ. & RIKEN IMS Workshop, Yokohama, Japan. August, 2014.

Laboratory for Skin Homeostasis

Team Leader: Masayuki Amagai

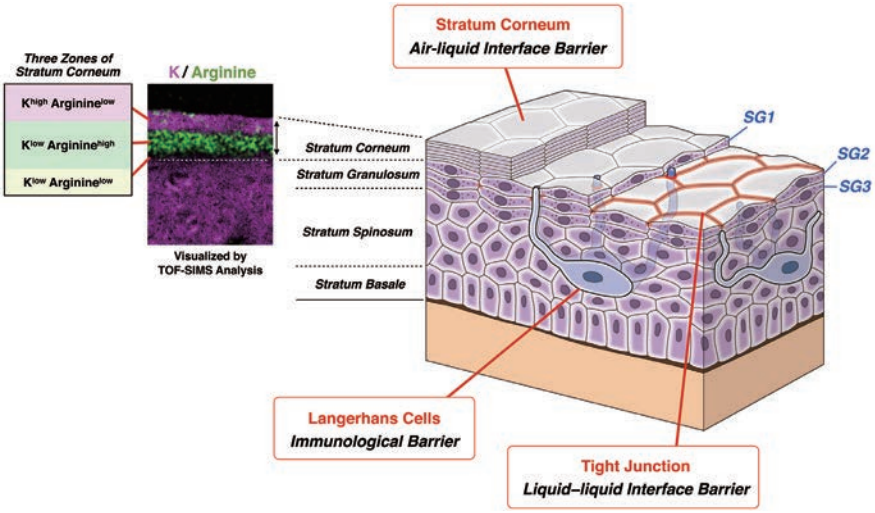


Figure: Barrier components of the skin, where immunity meets external antigens.
Three major barrier components of the epidermis: stratum corneum, tight junctions, and the Langerhans cell network as an immunological barrier. The stratum corneum has at least thee layers with different functions, as visualized by TOF-SIMS (Time of flight secondary ion mass spectrometry) (Kubo et al., Sci Rep, 2013).

When the immune system encounters external antigens in the skin, it tends to react to them. In contrast, when the immune system encounters antigens in the gut, it tends to tolerate them. However, the exact mechanisms for these opposing immune reactions are still largely unknown. Our laboratory attempts to dissect and understand the skin as an immune organ. In particular, we are studying skin barrier formation, function, and its dysfunction in atopic diseases.

Skin is composed of three components: epidermis, dermis and subcutaneous fat tissue. Epidermis is a keratinized stratified squamous epithelium and forms an effective barrier, which is essential for the prevention of the invasion of microorganisms, chemical compounds and allergens into the body. The epidermis is composed of four distinct layers; stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG) and stratum corneum (SC), from bottom to top (Fig.).

Among many elements of the skin barrier, we are focusing on the SC as an air-liquid barrier, tight junctions as a liquid-liquid barrier, and the Langerhans cell network as an immunological barrier (Fig.). Tight junctions are formed in the second layer of the SG (SG2 cells) and Langerhans cells extend their dendrites above tight junctions to capture external antigens. Filaggrin deficiency, which is a predisposing factor for atopic disease, enhances penetration of the SC by external antigens.

The SC is 12 to 15 accumulated layers of corneocytes, which are terminally differentiated dead keratinocytes. Therefore, all the essential SC components are produced in SG1 cells. To understand the transcriptional activity of the SG layer, we have performed RNA-seq analysis of mouse SG layer cells and identified various differentiation-specific genes. We also started to perform electron microscopic visualization of the SG and SC layers. Based on these analysis, we are planning to interpret cell biological changes in SG cells at the onset of dermatitis in several dermatitis model mice.

Recent Major Publications

Yoshida K, Kubo A, Fujita H, Yokouchi M, Ishii K, Kawasaki H, Nomura T, Shimizu H, Kouyama K, Ebihara T, Nagao K, Amagai M. Distinct behavior of human Langerhans cells and inflammatory dendritic epidermal cells at tight junctions in patients with atopic dermatitis. ***J Allergy Clin Immunol*** 134, 856–64 (2014)

Kubo A, Ishizaki I, Kubo A, Kawasaki H, Nagao K, Ohashi Y, Amagai M. The stratum corneum comprises three layers with distinct metal-ion barrier properties. ***Sci Rep*** 3, 1731 (2013)

Sasaki T, Shiohama A, Kubo A, Kawasaki H, Ishida Yamamoto A, Yamada T, Hachiya T, Shimizu A, Okano H, Kudoh J, Amagai M. A homozygous nonsense mutation in the gene for Tmem79, a component for the lamellar granule secretory system, produces spontaneous eczema in an experimental model of atopic dermatitis. ***J Allergy Clin Immunol*** 132, 1111–1120 (2013)

Invited Presentations

Amagai M. Towards antigen-specific immune suppression in pemphigus. Inflammatory Skin Disease Summit: The Translational Revolution, Vienna, Austria. November, 2014.

Amagai M. Clinical pictures as good teachers of basic science. 44th Annual Meeting of the European Society for Dermatological Research, Copenhagen, Denmark. September, 2014.

Matsui T. Functional Evolution of Mammalian Stratum Corneum by Retroposon-derived sequence. 16th Annual Meeting of Society for Evolutionary Studies, Japan, Osaka, Japan. August, 2014.

Amagai M. Skin as a site where immune system interacts with environment. RIKEN IMS-JSI International Symposium on Immunology 2014, Decoding Immune Complexity -Bench to Bedside-, Yokohama, Japan. June, 2014.

Amagai M. Epidermal barrier function and its dysfunction in atopic diseases. Singapore International Conference on Skin Research, Biopolis, Singapore. March, 2014.

Laboratory for Metabolic Homeostasis

Team Leader: Naoto Kubota

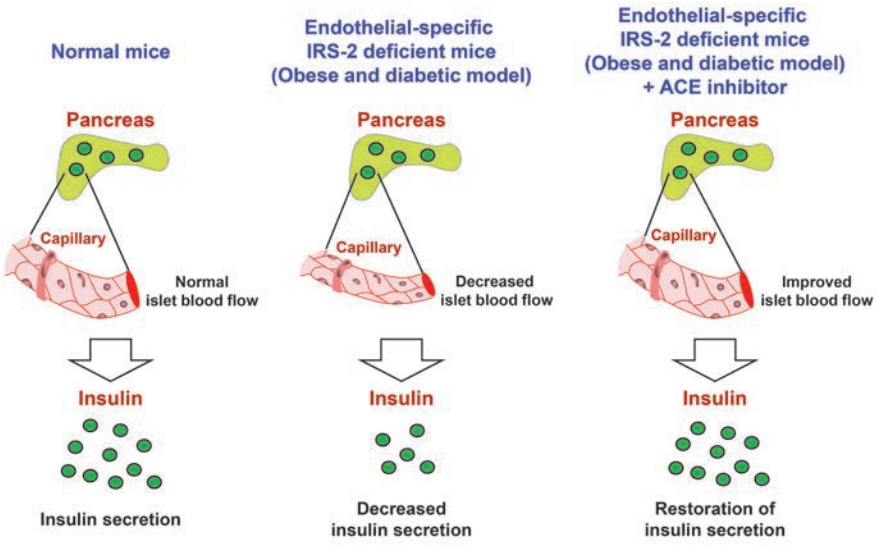


Figure: IRS-2 in the endothelial cells plays a crucial role in insulin secretion via islet blood flow
We have reported that IRS-2 expression levels in the endothelial cells reduced by hyperinsulinemia in obese and diabetic models. This reduction of IRS-2 expression levels also lead to obesity and type 2 diabetes. Endothelial-specific IRS-2 deficient (ETIrs2) mice is suitable as obese and diabetic models. Insulin secretion and islet blood flow decreased in ETIrs2 mice. ACE-inhibitor improved islet blood flow, resulting in restoration of insulin secretion. IRS-2 in the endothelial cells mediate insulin secretion through islet blood flow. Drugs for improvement of islet blood flow may be one of effective therapeutic strategy in obesity and type 2 diabetes subjects.

Recent Major Publications

Hashimoto S, Kubota N, Sato H, Sasaki M, Takamoto I, Kubota T, Nakaya K, Noda M, Ueki K, Kadowaki T. Insulin Receptor Substrate-2 (Irs2) in Endothelial Cells Plays a Crucial Role in Insulin Secretion. ***Diabetes*** 64, 876–86 (2015)

Takamoto I, Kubota N, Nakaya K, Kumagai K, Hashimoto S, Kubota T, Inoue M, Kajiwara E, Katsuyama H, Obata A, Sakurai Y, Iwamoto M, Kitamura T, Ueki K, Kadowaki T. TCF7L2 in mouse pancreatic beta cells plays a crucial role in glucose homeostasis by regulating beta cell mass. ***Diabetologia*** 57, 542–53 (2014)

Kubota T, Kubota N, Kadowaki T. The role of endothelial insulin signaling in the regulation of glucose metabolism. ***Rev Endocr Metab Disord*** 14, 207–16 (2013)

Invited Presentations

Kubota N. Pathology and treatment strategy for type 2 diabetes with obesity. Diabetes symposium 2014 in TOHOKU, Sendai, Japan. December, 2014.

Kubota N. Zonation-dependent selective insulin resistance of the liver in obesity and type 2 diabetes. The 8th Diabetes Leading-edge Conference, Chiba, Japan. August, 2014.

Kubota N. Molecular mechanisms of glucose and lipid metabolism. Meet the Expert of Diabetes 2014, Tokyo, Japan. June 2014.

Kubota N. Therapeutic strategy for type2 diabetes. Shinshu Basal Insulin Seminar, Nagoya, Japan. March, 2014.

In recent years there has been a rapid growth in the incidence of type 2 diabetes in both Western and Asian countries. This high prevalence is most likely the result of a complex interplay between genetic factors, such as reduced insulin secretion, and environmental factors, such as high-fat diet and decreased physical activity. However, the precise molecular mechanisms underlying the development and progression of type 2 diabetes remain unclear. The goal of our team is to identify molecular mechanisms of insulin secretion and insulin resistance.

Molecular mechanism of insulin secretion

Endothelial cells mediate blood flow, which is considered to be essential for insulin secretion. However, it is unclear whether endothelial cells are involved in the regulation of insulin secretion. We examined the relationship between insulin secretion and endothelial cells using the endothelial-specific Insulin Receptor Substrate-2 (IRS-2) knockout (ETIrs2KO) mice. Although insulin secretion from isolated islets was maintained, insulin secretion was significantly impaired in the ETIrs2KO mice. The islet blood flow was also significantly reduced these mice. Enalapril treatment, an ACE-inhibitor, improved the islet blood flow, resulting in the restoration of insulin secretion in the ETIrs2KO mice. These data suggest that IRS-2 in the endothelial cells regulates islet blood flow, mediating insulin secretion (Diabetes, in press).

Molecular mechanism of insulin resistance

The liver plays an important role in the control of glucose homeostasis. Interestingly, in patients with type 2 diabetes and obesity, hyperglycemia and hepatic steatosis often coexist. This would seem to indicate that the insulin signaling pathway is impaired in gluconeogenesis but preserved in lipogenesis. This phenomenon is referred to as “selective insulin resistance” and has recently received increasing attention. To address “selective insulin resistance” in the liver, we have been focusing our research on IRS-1 and IRS-2.

Laboratory for
Immune Crosstalk

Team Leader: Hilde Cheroutre

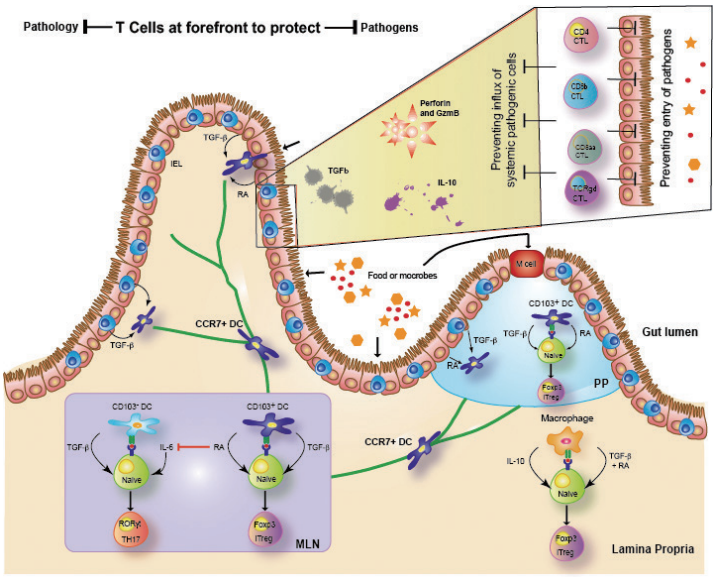


Figure: Epithelial T cells (epiT cells) protect the mucosal barrier of the intestine from pathogen- and inflammation-induced pathology.
T cells residing within the epithelium of the intestine are phenotypically heterogeneous but they are all specialized to protect the mucosal barrier against pathogen- and immune cell-induced pathology. In contrast to the T cells in the periphery, epiT cells are antigen-experienced T cells that encountered their antigen initially during selection in the thymus (agonist selected CD8aa TCRαβ and TCRγδ precursor cells) or as mature cells in the periphery (CD8αβ CTL and CD4 CTL). Although the various epiT cell subsets display different antigen specificity and MHC restriction and although they follow different paths of effector cell differentiation, they all acquire cytolytic and regulatory capacity. The functional specialization of epiT cell adapts them to provide optimal protection in the face of preserving the integrity of the delicate mucosal barrier.

Recent Major Publications

Vicente-Suarez I, Larange A, Reardon C, Matho M, Feau S, Chodaczek G, Park Y, Obata Y, Gold R, Wang-Zhu Y, Lena C, Zajonc DM, Schoenberger SP, Kronenberg M, Cheroutre H. Unique lamina propria stromal cells imprint the functional phenotype of mucosal dendritic cells. ***Mucosal Immunol*** 8, 141–51 (2015)

Mayans S, Stepniak D, Palida SF, Larange A, Dreux J, Arlian BM, Shinnakasu R, Kronenberg M, Cheroutre H, Lambolez F. αβT cell receptors expressed by CD4(-)CD8αβ(-) intraepithelial T cells drive their fate into a unique lineage with unusual MHC reactivities. ***Immunity*** 41, 207–18 (2014)

Fu G, Casas J, Rigaud S, Rybakin V, Lambolez F, Brzostek J, Hoerter JA, Paster W, Acuto O, Cheroutre H, Sauer K, Gascoigne NR. Themis sets the signal threshold for positive and negative selection in T-cell development. ***Nature*** 504, 441-5 (2013)

Invited Presentations

Cheroutre H. Mucosal T cells: Same Players Different Strategies. UCLA 13T Immunology Forum Seminar Series, Los Angeles, USA. December, 2014.

Cheroutre H. How the Gut Primes the Immune System. Annual Scientific Meeting of American College of Rheumatology (ACR), Boston, USA. November, 2014.

Cheroutre H. Mucosal Immunity: Taking Strategic Planning a Step Further. The Dutch Society for Immunology Annual Symposium, "Mucosal Immunity", Lunteren, the Netherlands. April, 2014.

Cheroutre H. New Emerging Transcription Factor and Cytokine Networks at the Mucosal Interface of the Intestine. Keystone Symposium on Molecular and Cellular Biology: Emerging Cytokine Networks (J3). Session: Regulation of Tissue-Resident ILC and T Cells, Vancouver, Canada. January, 2014.

Our research continues to elucidate mechanisms of mucosal immune protection and regulation. In a recent study, we uncovered an unexpected degree of plasticity for CD4 T helper (Th) cells which, upon antigenic stimulation, are able to terminate the expression of the Th transcription factor, ThPOK, and differentiate into cytotoxic T lymphocytes (CTL). At steady state, CD4 CTLs remain quiescent and express a self-regulated phenotype. However under challenging conditions, these cells have the potential to transform into potent inflammatory killer effector cells (Mucida et al., Nat Immunol, 2013).

Overall, based on the insights we are gaining from our research, a clear picture has begun to emerge showing that the immune defense of the intestine adapts to the local environment and specializes to provide the most efficient and immediate protection in the face of preserving the integrity of the most critical mucosal barrier of the body.

In another study, we are aiming to understand the various mechanisms and processes that lead to Central Tolerance. Our previous research showed that in addition to conventional selection, a process of so called “agonist” selection operates in the thymus and preserves self-specific thymocytes and functionally differentiates these precursor cells into beneficial pre-programmed protective or regulatory T cells. In an effort to understand what factors control the decisive checkpoint during thymic selection, we identified “Themis” as a critical switch. By making various kinds of mutant Themis knock-in mice, we found that localization of Themis protein in the nucleus is critical for its function. In addition to the molecular mechanisms of Themis in the nucleus, we are now trying to elucidate the molecular and cellular factors and events that connect the Pre-TCR and TCR signal strength with thymic selection and the fate decision of the developing T cells.

Laboratory for Inflammatory Regulation

Team Leader: Takashi Tanaka

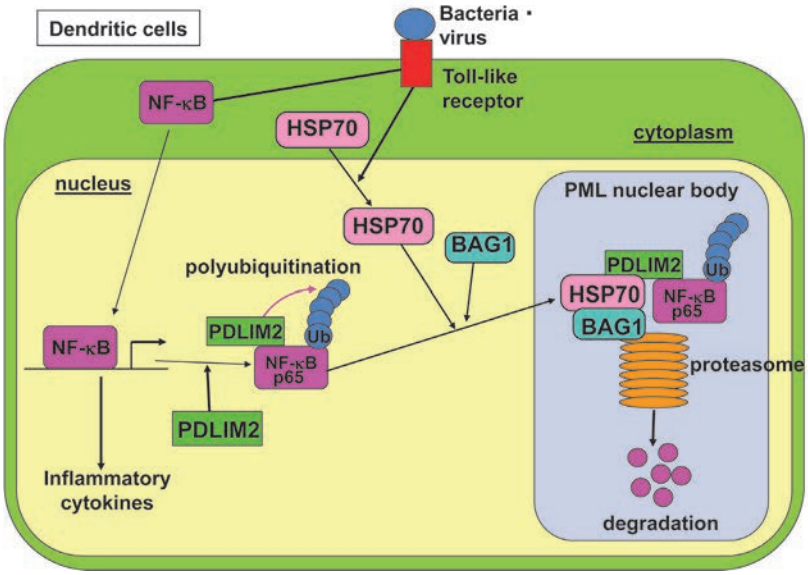


Figure: HSP70 is essential for PDLIM2-mediated degradation of NF-κB p65.
PDLIM2 binds to the p65 subunit of NF-κB and promotes p65 polyubiquitination in the nucleus. PDLIM2 then targets p65 into discrete intranuclear compartments, called PML nuclear bodies. HSP70 binds to PDLIM2 and facilitates delivery of the NF-κB-PDLIM2 complex to the proteasome cooperatively with BAG1. Polyubiquitinated p65 is ultimately degraded by the proteasome in PML nuclear bodies.

The inflammatory response is an important host defense mechanism to sense and eliminate invading microbial pathogens. Dendritic cells first detect pathogens and activate the transcription factor NF-κB, which enters the nucleus and induces the expression of a series of inflammation-related genes. These initially helpful inflammatory responses must be terminated at the appropriate time point, otherwise excessive responses can damage normal tissue and may cause autoimmune diseases. Our research goal is to identify key regulators of inflammation-related signal transduction pathways and to clarify the complete picture of the molecular mechanisms for regulating inflammatory responses. We previously identified PDLIM2 (PDZ and LIM domain protein-2), a nuclear protein that belongs to a large family of LIM proteins, as one of the key factors negatively regulating inflammatory responses. We demonstrated that PDLIM2 negatively regulates NF-κB activity and subsequent inflammatory responses, acting as a nuclear ubiquitin E3 ligase targeting the p65 subunit of NF-κB. (Tanaka T, Nat Immunol, 2007). We have recently studied how PDLIM2-mediated p65 degradation is controlled and found that heat shock protein 70 (HSP70), a molecular chaperone, is required for PDLIM2 to degrade p65 and suppress NF-κB activation following inflammatory responses. In dendritic cells, HSP70 is detected only in the cytoplasm without stimulation, but it is translocated to the nucleus after TLR stimuli. HSP70 then associated with both PDLIM2 and BAG-1, a proteasome-associated protein, and promoted the transport of the NF-κB-PDLIM2 complex to the proteasome, thereby facilitating p65 degradation. Consistently, either HSP70 deficiency or BAG-1 knockdown in dendritic cells leads to increased nuclear p65 protein levels and thus enhanced production of proinflammatory cytokines in response to TLR stimuli (Tanaka T, Sci Signal, 2014). These studies should contribute to our understanding of the pathogenesis of human autoimmune diseases and provide novel targets to develop new treatments for these diseases.

Recent Major Publications

Tanaka T, Shibazaki A, Ono R, Kaisho T. HSP70 mediates degradation of the p65 subunit of nuclear factor κB to inhibit inflammatory signaling. ***Sci Signal*** 7, ra119 (2014)

Yamazaki C, Sugiyama M, Ohta T, Hemmi H, Hamada E, Sasaki I, Fukuda Y, Yano T, Nobuoka M, Hirashima T, Iizuka A, Sato K, Tanaka T, Hoshino K, Kaisho T. Critical roles of a dendritic cell subset expressing a chemokine receptor, XCR1. ***J Immunol*** 190, 6071–82 (2013)

Invited Presentations

Tanaka T. From Molecules to Diseases. Clarification of the molecular mechanisms that negatively regulate inflammatory responses and association analysis of autoimmune diseases by GWAS. The 3rd Akashi-cho rheumatic collaborative seminar in St Luke's International Hospital, Tokyo, Japan. October, 2014.

Tanaka T. Negative regulation for inflammatory responses and its association with autoimmune diseases. Monash Univ. & RIKEN IMS Workshop, Yokohama, Japan. August, 2014

Laboratory for Cytokine Regulation

Team Leader: Masato Kubo

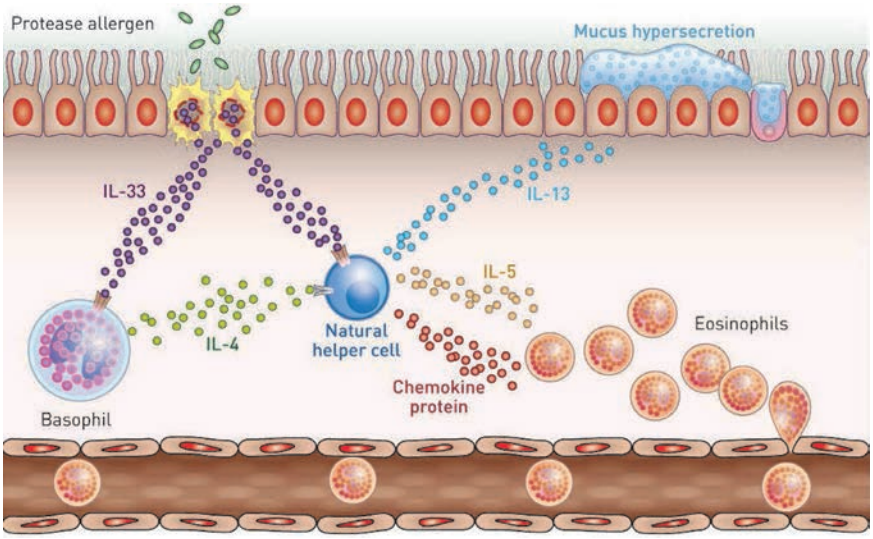


Figure: Cross-talk of basophils and group 2 innate lymphoid cells (ILC2s)/Natural helper (NH) cells in asthmatic responses
Cysteine protease allergen induced IL-4 from basophils, and the basophil-derived IL-4 has a critical role in the secretion of IL-5, IL-13 and CCL11 from ILC2s/NH cells that lead to T-independent asthmatic airway inflammation.

Allergy is thought to be controlled by type 2 cytokines, including IL-4, IL-5, and IL-13. IL-4 promotes IgE production by B cells, IL-5 induces development, recruitment, and activation of eosinophils, and IL-13 is tightly associated with multiple events in the effector phase of allergic responses, inducing airway hyper-responsiveness and hyperproduction of mucus. Recently, these type 2 cytokines were found to be secreted not only by TH2 cells but also by innate immune cells, including mast cells, basophils, eosinophils, and group 2 innate lymphoid cells (ILC2s), which have been discovered in the gut-associated mucosal tissues.

Allergic asthma is a chronic inflammatory disease characterized by airway obstruction and wheezing. Conventional mouse models have illustrated the importance of TH2 cells and eosinophilic inflammation and, more recently the importance of basophils and ILC2s. Asthmatic inflammation is inducible by allergen proteases, including those from papain and house dust mites (HDM). The HDM-derived protease Derp1 causes the airway influx of eosinophils and bronchoconstriction in asthma patients. Papain a plant-derived cysteine protease, causes occupational asthma. To examine the role of basophils and mast cells, we established a diphtheria toxin-based conditional deletion system using an *Il4* enhancer that we previously found was specific for IL-4 production by mast cells or basophils (Mas-TRECK and Bas-TRECK mice). Conditional deletion of basophils caused a resolution of papain-induced eosinophilia and mucus production. Resolution of eosinophilia was also observed in mice lacking IL-4 production specifically in basophils, indicating that basophil-derived IL-4 not only enhanced expression of CCL11, IL-5, IL-9 and IL-13 by natural helper (NH) cells, thus attracting eosinophils, but also promoted expansion of these ILC2s. These results demonstrate that IL-4 from basophils has an important role in the IL-33-NH cell-cytokine/chemokine axis, subsequently leading to protease allergen-induced airway inflammation.

Recent Major Publications

Motomura Y, Morita H, Moro K, Nakae S, Artis D, Koyasu S, Kubo M. Basophil-derived interleukin-4 controls the function of natural helper cells, a member of ILC2s, in lung inflammation. ***Immunity*** 40, 758–71 (2014)

Kurashima Y, Amiya T, Fujisawa K, Shibata N, Suzuki Y, Kogure Y, Hashimoto E, Otsuka A, Kabashima K, Sato S, Sato T, Kubo M, Akira S, Miyake K, Kunisawa J, Kiyono H. The enzyme Cyp26b1-mediates Inhibition of mast cell activation by fibroblasts to maintain skin-barrier homeostasis. ***Immunity*** 40, 530–41 (2014)

Noti M, Wojno ED, Kim BS, Siracusa MC, Giacomin PR, Nair MG, Benitez AJ, Ruymann KR, Muir AB, Hill DA, Chikwava KR, Moghaddam AE, Sattentau QJ, Alex A, Zhou C, Yearley JH, Menard-Katcher P, Kubo M, Obata-Ninomiya K, Karasuyama H, Comeau MR, Brown-Whitehorn T, de Waal Malefyt R, Sleiman PM, Hakonarson H, Cianferoni A, Falk GW, Wang ML, Spergel JM, Artis D. Thymic stromal lymphopoietin-elicited basophil responses promote eosinophilic esophagitis. ***Nat Med*** 19, 1005–13 (2013)

Invited Presentations

Kubo M, Role of T follicular helper cells in influenza virus protection. France-Japan Immunology meeting, Cassis, France. October, 2014.

Kubo M, Notch regulates reciprocal expression of CCR7 versus CXCR5 to control central memory T cell generation. The 2nd Symposium of International Immunological Memory and Vaccine Forum, La Jolla, USA. August, 2014.

Kubo M, Regulation of allergic airway inflammation by basophil and innate lymphoind cells. The 79th Annual Meeting of the Japanese Society of Interferon & Cytokine Research, Sapporo, Japan. June, 2014.

Kubo M, Understanding a role of cytokine signaling in homeostatic skin regulation. Shanghai Immunodermatology Forum 2014, Shanghai, China. May, 2014.

Kubo M, Regulation of allergic airway inflammation by basophil and innate lymphoind cells. Dry-eye allergy joint seminer, Tokyo, Japan. January, 2014.Conference on Skin Research, Biopolis, Singapore. March, 2014.

Core for Precise Measuring and Modeling

Photo: The new mass spectrometry laboratory
To enhance metabolomic and proteomic analyses in IMS, new laboratory space dedicated to mass spectrometry was created.

Toward the ultimate goal of obtaining a comprehensive understanding of the pathogenesis of human diseases, the functions of the Core for Precise Measuring and Modeling are three pronged: production of mouse models, multiomics measurements and quantitative bioimaging, and bioinformatics/modeling of human disease processes. Through close interactions among these three branches of the core, we aim to collect a wide variety of quantitative data in order to build a computational and predictive network of the disease process. As for the production of genetically engineered mice that will be used as models of human diseases, the laboratory for Developmental Genetics has begun to apply recent advances in genome engineering technology, e.g., CRISPR/Cas9-based genome editing, and thereby considerably enhance the production capacity and power of the disease models. Regarding the precise quantitative measurements branch, one focus in 2014 has been to enhance the power of metabolite analysis by the Laboratory for Metabolomics (Photo). Together with mRNA/protein profiling by the Laboratory for Integrative Genomics, the enhanced multiomics measurements and bio imaging (Laboratory for Tissue Dynamics) will greatly contribute to exploration of the etiology of human diseases. After being processed by bioinformatics (Laboratory for Integrated Bioinformatics), the datasets are used for modeling (Laboratories for Disease Systems Modeling and Integrated Cellular Systems). As a leading IMS project, an atopic dermatitis model mouse, provided by the Laboratory for Immunogenetics, has been extensively analyzed from several different angles, fully exploiting the analysis powers of this core. These efforts should enable us to identify new biomarkers for early diagnosis and prevention of atopic dermatitis in the very near future.

Laboratory for Developmental Genetics

Group Director: Haruhiko Koseki

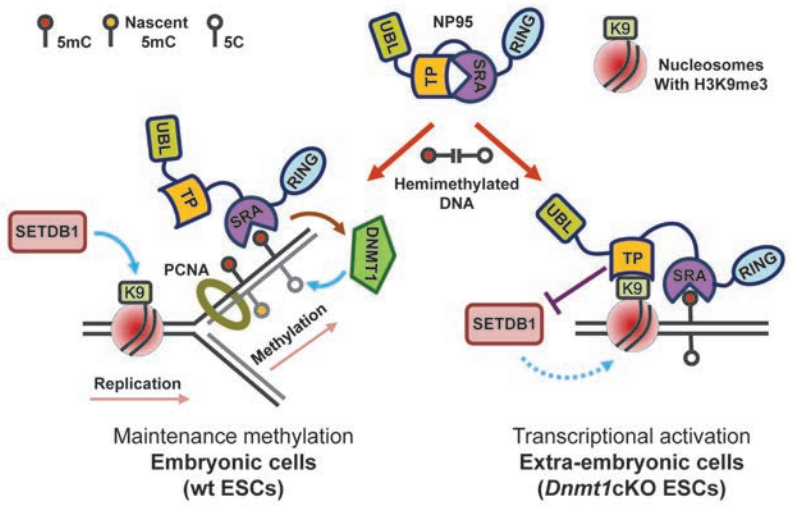


Figure: Role of NP95 in embryonic and extra-embryonic tissues
Without substrate (i.e. hemimethylated DNA), the NP95 SRA (SET and RING-finger associated) domain interacts with the neighboring TP (Tudor and PHD), assuming a mutually inhibitory conformation (top). Hemimethylated DNA (5-cytosine methylation of only one strand of the CpG dyad) binding by NP95-SRA elicits two different downstream pathways. In embryonic cells, NP95 recruits DNMT1- the major DNA methyltransferase, to maintain methylation upon DNA replication (left). Association of NP95 with naturally occurring hemimethylated DNA in extra-embryonic cells results in transcriptional activation through inhibition of the SETDB1 (H3K9 trimethyltransferase) and H3K9me3-mediated repressive machinery (right).

The Developmental Genetics Research Group focuses on the epigenetic regulation of organ development and stem cell functions by Polycomb group (PcG) proteins and molecules that mediate DNA methylation.

Chromatin dynamics associated with activation of Polycomb-repressed genes

How PcG-repressed genes are activated upon receipt of developmental cues is poorly understood. We used the *Meis2* locus as a model to identify the role of a tissue-specific enhancer in displacing the PcG complex from the promoter. *Meis2* repression in early development depends on the binding of RING1B, an essential component of PcG, to the promoter. This is coupled with the association of the promoter with another RING1B-binding site (RBS) at the 3' end of the *Meis2* locus. During early midbrain development, a midbrain-specific enhancer (MBE) transiently associates with the promoter/RBS, forming a promoter/MBE/RBS tripartite interaction in a RING1-dependent manner. Subsequently, the RING1B-bound RBS dissociates from the tripartite, leaving promoter/MBE engagement to activate *Meis2* expression. This study therefore demonstrates the role of PcG and/or related factors in *Meis2* activation by regulating the topological transition of cis-regulatory elements.

Regulation of endogenous retroviral elements (ERVs) by the SRA protein NP95

ERVs are abundant in the mammalian genome and are transcriptionally silenced by repressive epigenetic marks such as DNA methylation and Histone H3 Lysine 9 (H3K9) methylation. However, during specific windows, such as early embryogenesis and primordial germ cell (PGC) development, DNA methylation is globally erased, which potentially exposes the genome to aberrant activation of ERVs. We have found that functional inhibition of the SRA protein NP95, an essential component of the maintenance methylation machinery, could mediate global demethylation and at the same time ensure silencing of ERV sequences, particularly in the embryo proper. Absence of NP95 facilitates the transition from a DNA methylation-dependent state to an H3K9 methylation-dependent state contributing to ERV silencing. Paradoxically, NP95 also has a role in transcribing ERV sequences in the extra-embryonic tissues, such as the placenta, under normal physiological conditions, likely by destabilizing the H3K9 methylation pathway. Taken together, our observations reveal that the fidelity of histone methylation-dependent proviral silencing is dependent upon timely maintenance DNA methylation.

Recent Major Publications

Obata Y, Furusawa Y, Endo TA, Sharif J, Takahashi D, Atarashi K, Nakayama M, Onawa S, Fujimura Y, Takahashi M, Ikawa T, Otsubo T, Kawamura YI, Dohi T, Tajima S, Masumoto H, Ohara O, Honda K, Hori S, Ohno H, Koseki H, Hase K. The epigenetic regulator Uhrf1 facilitates the proliferation and maturation of colonic regulatory T cells. ***Nat Immunol*** 15, 571–9 (2014)

Blackledge NP, Farcas AM, Kondo T, King HW, McGouran JF, Hanssen LL, Ito S, Cooper S, Kondo K, Koseki Y, Ishikura T, Long HK, Sheahan TW, Brockdorff N, Kessler BM, Koseki H, Klose RJ. Variant PRC1 complex-dependent H2A ubiquitylation drives PRC2 recruitment and polycomb domain formation. ***Cell*** 157, 1445–59 (2014)

Kondo T, Isono K, Kondo K, Endo TA, Itohara S, Vidal M, Koseki H. Polycomb potentiates meis2 activation in midbrain by mediating interaction of the promoter with a tissue-specific enhancer. ***Dev Cell*** 28, 94–101 (2014)

Invited Presentations

Koseki H. iPS-mediated induction of human NKT cells and their application for cancer therapy. The 35th Annual Meeting of the Japanese Society of Inflammation and Regeneration, Okinawa, Japan. July, 2014.

Laboratory for Integrative Genomics

Group Director: Osamu Ohara

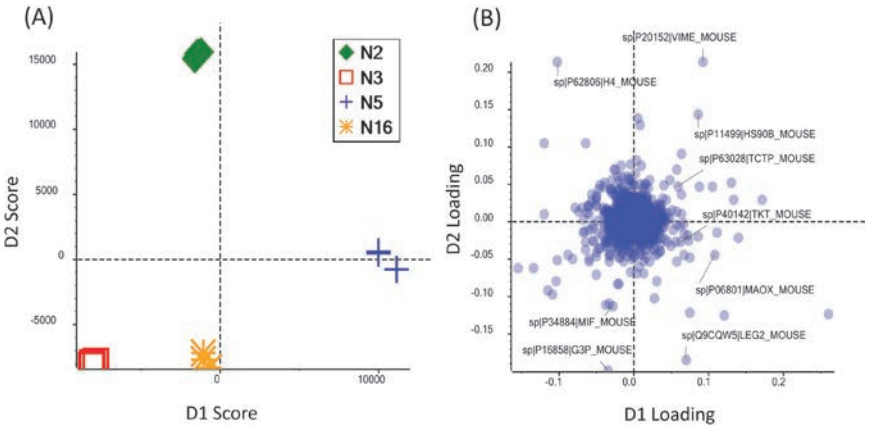


Figure: Principal Component Analysis (PCA) of protein profiles in four types of mouse tissues (N2, N3, N5, N16)
A: PCA score plot shows separate clusters of four types of mouse tissues at different disease states. B: PCA loading plot shows the proteins responsible for separation of the groups in the corresponding score plot.

Although "genomics" itself has always been an integrative science, our laboratory name is "Integrative Genomics" to indicate and emphasize our intention to gain new insights into regulation of complex biological systems by amalgamating spatio-temporal multi-scale data. Thus, besides our central support activities and involvement in collaborative/strategic programs at IMS, we have placed technology development as a central mission of my group. In this regard, our current technology interest is in development of methods to compare and/or link macroscopic genomic data with data at the single-cell resolution. Recent publications regarding measurements of protein secretion from single cells are examples of the outcomes of our efforts in this direction. To further enhance such research activities, we think that the technology of absolute quantification of biomolecules would significantly increase its importance and have begun to implement such technologies at the protein level. SWATH (sequential window acquisition of all theoretical fragment ion spectra) is one such technology for high throughput, label-free protein quantification based on mass spectrometry. However, to link SWATH data with the corresponding single-cell protein profiles, we need high-quality antibodies for detection of specific proteins of interest as demonstrated in our recent publication (Shirasaki et al., Sci Rep, 2014). We are thus working on technology development of high-throughput production of recombinant antibodies with specified affinity/specificity. The combination of mass spectrometry-based and affinity-based proteomics approaches will enable us to open a new avenue leading to real spatio-temporal and multi-scale data at the protein level.

Recent Major Publications

Aoki Y, Watanabe T, Saito Y, Kuroki Y, Hijikata A, Takagi M, Tomizawa D, Eguchi M, Eguchi-Ishimae M, Kaneko A, Ono R, Sato K, Suzuki N, Fujiki S, Koh K, Ishii E, Shultz LD, Ohara O, Mizutani S, Ishikawa F. Identification of CD34+ and CD34- leukemia-initiating cells in MLL-rearranged human acute lymphoblastic leukemia ***Blood*** 125, 967–80 (2015)

Liu T, Yamaguchi Y, Shirasaki Y, Shikada K, Yamagishi M, Hoshino K, Kaisho T, Takemoto K, Suzuki T, Kuranaga E, Ohara O, Miura M. Single-cell imaging of caspase-1 dynamics reveals an all-or-none inflammasome signaling response. ***Cell Rep*** 8, 974–82 (2014)

Shirasaki Y, Yamagishi M, Suzuki N, Izawa K, Nakahara A, Mizuno J, Shoji S, Heike T, Harada Y, Nishikomori R, Ohara O. Real-time single-cell imaging of protein secretion. ***Sci Rep*** 4, 4736 (2014)

Invited Presentations

Ohara O. Construction of A Clinical Sequencing Pipeline for Genetic Diseases. The 59th Annual Meeting of the Japan Society of Human Genetics/The 21st Japanese Society for Gene Diagnosis and Therapy, Tokyo, Japan. November, 2014.

Hijikata A, Ohara O. Structural Bioinformatics of Human Genetic Diseases. The 86th Annual Meeting of The Genetics Society of Japan, Shiga, Japan. September, 2014.

Ohara O. Innovation and Invention in Genomics. 18th Meeting of Molecular Composite Medicine, Osaka, Japan. July, 2014.

Ohara O. What is "Next-Generation DNA Sequencing" for? Liaison Laboratory regular seminar FY2014, Kumamoto, Japan. May, 2014.

Ohara O. Implementation of High-Volume Genomic Analyses by Microfluidics/microchip Technologies: Towards Integrative Medical Sciences for Preventive Medicine. International Conference on Electronics Packaging 2014, Toyama, Japan. April, 2014.

Laboratory for Disease Systems Modeling

Group Director: Hiroaki Kitano

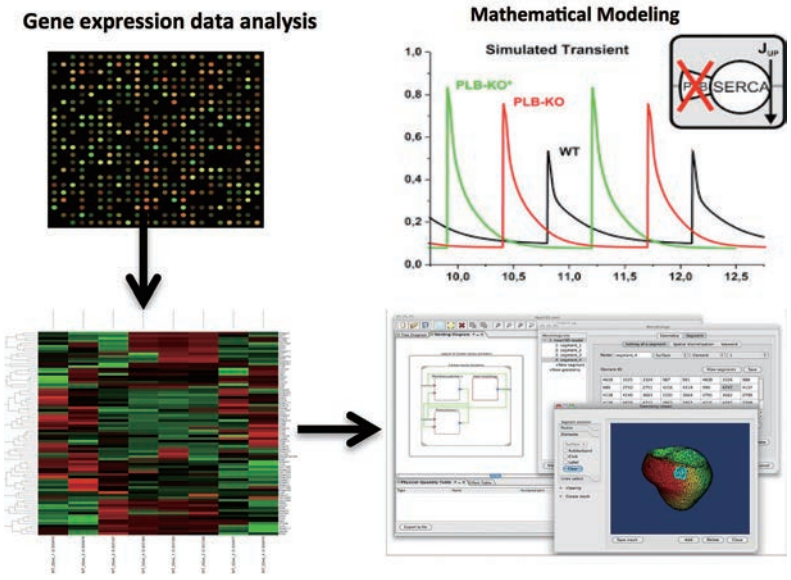


Figure: Systems level approaches to investigate atopic dermatitis.
The LDSM is investigating systems-level principles underlying diseases and developing multi-layer physiological models and detailed cellular and molecular interaction models by using gene expression data.

Understanding systems-level mechanisms of Atopic Dermatitis (AD) and supporting computational systems biology needs of the center are missions of the LDSM.

During 2014, the LDSM has made significant progress in developing basic analysis pipelines for RNA-seq analysis, network reconstruction, and dynamical modeling of AD, focusing on time series analysis involving pre- and post-onset of the disease.

On the technology development front, we have developed (1) a basic mRNA-seq analysis pipeline tailored for IMS facilities, (2) a preliminary text-mining system for model reconstruction, (3) a genomics-systems biology integrated analysis pipeline using Galaxy and Garuda platforms, etc. These have already proven to be useful for analyses and reproducibility. In the future, we will continue to improve and expand this technology infrastructure.

In terms of data analysis and modeling, we have completed an initial analysis of mRNA data provided by experimental IMS teams and identified a set of critical genes that are implicated in a subset of AD outbreaks. We have also created a tissue specific gene analysis pipeline by using heterogeneous in-house experimental data that are important in AD.

We have also conducted our own cell-based experiments to further validate our findings and to provide missing information. In parallel, we have developed a computational model of AD, initially focusing on keratinocytes. This model will be integrated with other models developed by Dr. Mariko Okada (RIKEN) and Dr. Reiko Tanaka (ICL) to create a more comprehensive model of AD.

On the experimental side, we have established a mammalian cell-based experimental facility to verify a series of hypotheses derived from the model prediction, as well as a comprehensive siRNA screening system to identify possible roles of miRNA in AD. In addition, a yeast gTOW system is now up-and-running to generate robustness-profiles to develop high precision yeast cell cycle models that should eventually contribute to understanding the fragility of mammalian cellular homeostasis implicated in AD and other diseases.

Recent Major Publications

Watanabe T, Kawakami E, Shoemaker JE, Lopes TJ, Matsuoka Y, Tomita Y, Kozuka-Hata H, Gorai T, Kuwahara T, Takeda E, Nagata A, Takano R, Kiso M, Yamashita M, Sakai-Tagawa Y, Katsura H, Nonaka N, Fujii H, Fujii K, Sugita Y, Noda T, Goto H, Fukuyama S, Watanabe S, Neumann G, Oyama M, Kitano H, Kawaoka Y. Influenza virus-host interactome screen as a platform for antiviral drug development. ***Cell Host Microbe*** 16, 795–805 (2014)

Marti-Solano M, Birney E, Bril A, Della Pasqua O, Kitano H, Mons B, Xenarios I, Sanz F. Integrative knowledge management to enhance pharmaceutical R&D. ***Nat Rev Drug Discov*** 13, 239–40 (2014)

Hsin KY, Ghosh S, Kitano H. Combining machine learning systems and multiple docking simulation packages to improve docking prediction reliability for network pharmacology. ***PLos One*** 8, e83922 (2013)

Invited Presentations

Kitano H. Making comprehensible maps from comprehensive knowledge - New approaches to information and knowledge management. Luxembourg, Life Science Hub in Europe, Tokyo, Japan. October, 2014.

Kitano H. Systems Biology and Applications. ICSB 2014, Melbourne, Australia. September, 2014.

Laboratory for Immunogenetics

Team Leader: Hisahiro Yoshida

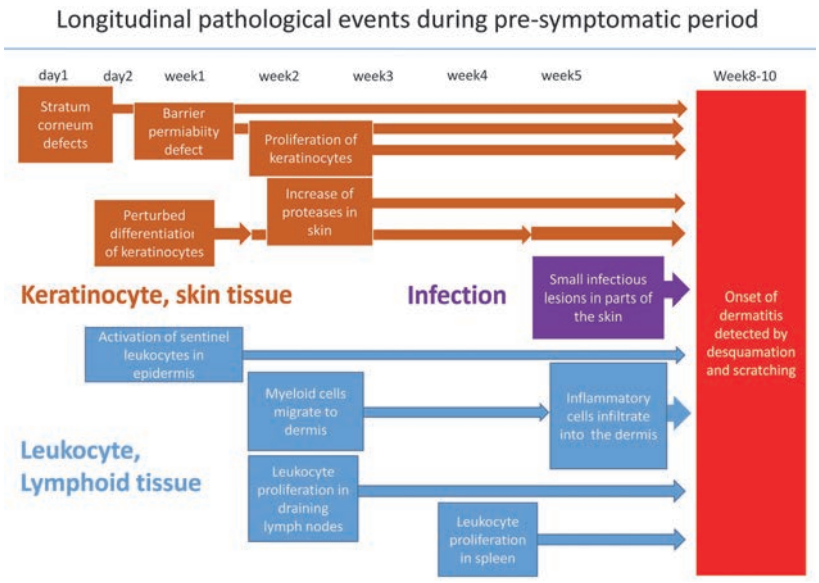


Figure: Pre-symptomatic disease development in the Spade mutant
Pre-symptomatic events occurring in the *Spade* mutant were detected by histological, cytological and molecular biological analysis and are summarized in the figure as longitudinal steps. The pre-symptomatic events occurred first in epidermal tissues, and this was followed by local immune activation, local proteases expression, migration of innate immune cells to the lesion, microbial infection, and finally by a systematic immune reaction.

Before the onset of an inflammatory disease, there must be an accumulation of many imperceptible pathogenic events in the human body as part of pre-symptomatic disease development under the influence of genetic and environmental factors. If one could precisely monitor and understand these pre-symptomatic longitudinal multiple events occurring in a healthy individual, it should be possible to predict the timing of disease onset and to take measures to prevent it. This strategy will be beneficial for many of us in the coming era when everyone can know their own genome sequence information and genetic risk factors shortly after birth.

In our laboratory, we had been working to identify the genetic factors for allergic or immune disease development by phenotype screening of chemical mutagen, N-ethyl N-nitrosourea (ENU), -induced mutant mice on a C57BL/6J background. Among the mutants identified, we have focused on a dermatitis model in which the disease develops at approximately 8 weeks after birth, as detected by ear skin desquamation and scratching of the skin a few days after the initial symptoms. This phase is followed by a Th2 biased immune response detected by serum IgE, IgG1 and elevated histamine levels 3 weeks later and then, 8 weeks after that, a Th1 immune bias ensues, with elevated serum IgG2b and IgG2c. At this final stage, the pathological findings led to the diagnosis of a chronic inflammatory condition of the skin. These symptoms are in part compatible with those of human atopic dermatitis. Therefore we named this mutant *Spade* (Stepwise progressive atopic dermatitis).

Using this disease model, we have precisely analyzed the pre-symptomatic pathological events as shown in the figure. The accumulation of all these events leads to the onset of dermatitis in the late stage. Now we are trying to identify the molecular networks responsible for each of these pre-symptomatic events.

Recent Major Publications

Obata Y, Kimura S, Nakato G, Iizuka K, Miyagawa Y, Nakamura Y, Furusawa Y, Sugiyama M, Suzuki K, Ebisawa M, Fujimura Y, Yoshida H, Iwanaga T, Hase K, Ohno H. Epithelial-stromal interaction via Notch signaling is essential for the full maturation of gut-associated lymphoid tissues. ***EMBO Rep*** 15, 1297–304 (2014)

Miyai T, Hojyo S, Ikawa T, Kawamura M, Irie T, Ogura H, Hijikata A, Bin BH, Yasuda T, Kitamura H, Nakayama M, Ohara O, Yoshida H, Koseki H, Mishima K, Fukada T. Zinc transporter SLC39A10/ZIP10 facilitates antiapoptotic signaling during early B-cell development. ***Proc Natl Acad Sci U S A*** 111, 11780—5 (2014)

Hojyo S, Miyai T, Fujishiro H, Kawamura M, Yasuda T, Hijikata A, Bin BH, Irie T, Tanaka J, Atsumi T, Murakami M, Nakayama M, Ohara O, Himeno S, Yoshida H, Koseki H, Ikawa T, Mishima K, Fukada T. Zinc transporter SLC39A10/ZIP10 controls humoral immunity by modulating B-cell receptor signal strength. ***Proc Natl Acad Sci U S A*** 111, 11786–91 (2014)

Invited Presentations

Yoshida H. Genetic factors and atopic dermatitis disease model. The 10th Tokyo Scientific Forum for Atopic Dermatitis and Psoriasis, Tokyo, Japan. December, 2014.

Yoshida H. Genetic factors and onset of atopic dermatitis in murine disease model. The 113th Annual Meeting of the Japanese Dermatological Association, Kyoto, Japan. May, 2014.

Laboratory for Integrated Bioinformatics

Team Leader: Todd D. Taylor

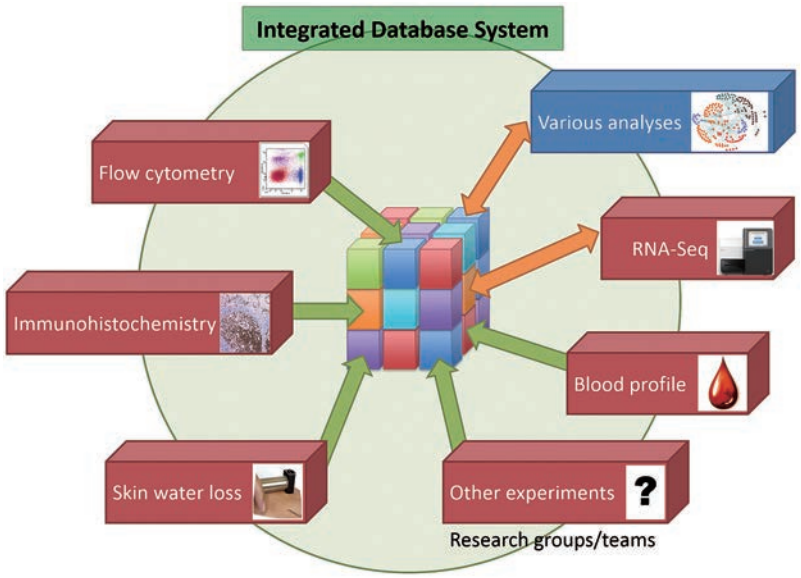


Figure: Integrated database and sample-tracking system
Various types of wet-lab experimental results, such as flow cytometry information, immunohistochemistry images, skin water loss measurements, and blood profiles (green arrows), will be input into the database directly from the respective labs. Other results, such as RNA-Seq data and analytical output (orange arrows), will be incorporated into the database using automated transfer protocols. All of the data will be available for viewing, tracking, downloading and analyzing by any authorized labs.

The main research focus of our laboratory is the development of computational tools and databases related to the analysis of metagenomic, and sometimes bacterial genomic, sequence data. In addition, we are also developing some general-purpose tools that are applicable to the wider research community.

Initially as part of a center project, we are developing an integrated database and sample-tracking system for the storage and distribution of massive and various types of experimental data, including: flow cytometry data, immunohistochemistry images, skin water loss measurements, blood profiles, RNA-Seq data, and so on. For maximum flexibility, this database is being designed not to be data-, project-, species-, or center- specific. We will also integrate other useful outside data resources. We hope to develop a secure, flexible system that makes it easy to manage, access, analyze, integrate, and visualize various data types as per user requirements.

We are also developing a revolutionary new web-based tool, called iCLiKVAL (http://iclikval.riken.jp/), that uses the power of crowdsourcing to add valuable annotation information to the rapidly accumulating volume of scientific literature (e.g., millions of PubMed citations and other media types), resulting in a comprehensive library of data that yields richer and more relevant literature search results. We will be actively promoting this resource in the coming year and we are strongly encouraging more and more scientists to get involved and contribute their valuable knowledge.

The ultimate goal of our research is to develop tools capable of efficiently processing and analyzing data from a variety of sources, with an emphasis on metagenomic data. With the advent of high-throughput next-generation sequencers, and subsequent massive data output, we anticipate the need for better means of visualizing and understanding the results and we will continue to put more emphasis in this critical area.

Recent Major Publications

Foong C, Lau NS, Deguchi S, Toyofuku T, Taylor TD, Sudesh K, Matsui M. Whole genome amplification approach reveals novel polyhydroxyalkanoate synthases (PhaCs) from Japan trench and nankai trough seawater. ***BMC Microbiol*** 14, 7. (2014) [Epub ahead of print]

International Glossina Genome Initiative. Genome sequence of the tsetse fly (Glossina morsitans): vector of African trypanosomiasis. ***Science*** 344, 380–6 (2014)

Jinda W, Taylor TD, Suzuki Y, Thongnoppakhun W, Limwongse C, Lertrit P, Suriyaphol P, Trinavarat A, Atchaneeyasakul LO. Whole exome sequencing in Thai patients with retinitis pigmentosa reveals novel mutations in six genes. ***Invest Ophthalmol Vis Sci*** 55, 2259–68 (2014))

Invited Presentations

Taylor TD. Introduction to metagenomics; Metadata management, sampling considerations, experimental approaches, workflow summary. CCB-USM Workshop on Metagenomics, Penang, Malaysia. December, 2014.

Kim S.-W. Microbial community analysis using 16S rRNA; BioRuby for beginners. CCB-USM Workshop on Metagenomics, Penang, Malaysia. December, 2014.

Laboratory for Tissue Dynamics

Team Leader: Takaharu Okada

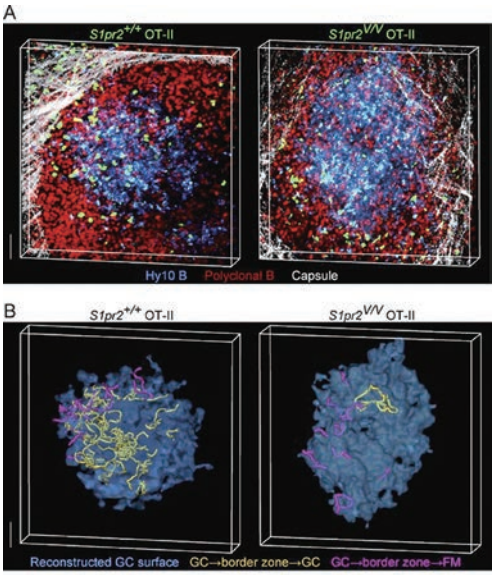


Figure: S1PR2 is important for Tfh cell retention in the GC.
(A) Two-photon imaging of S1PR2-sufficient (*S1pr2*$^{+/+}$) or S1PR2-deficient (*S1pr2*$^{V/V}$) OT-II Tfh cells (green) in the GC formed by Hy10 monoclonal B cells (blue) and the follicular mantle (FM), where polyclonal B cells (red) are accumulated. White are collagen fibers of lymph node capsules. (B) Cell tracking analysis of OT-II Tfh cells that access the interface zone from the GC. GC surfaces were reconstructed from the images in A. The tracks show migration paths of OT-II Tfh cells that entered the interface zone from the GC and then left the interface zone to the GC (yellow) or FM (pink).

The goal of the laboratory is to understand the mechanisms regulating cell migration and interactions in the tissues that shape adaptive immune responses. Currently, we have limited understanding of how generation of immunological memory and tolerance, two key features of adaptive immunity, are controlled by dynamic interactions among immune cells. For example, it is not understood how dynamics of B cells and helper T cells contribute to generation of humoral immune memory. As for cellular immunity mediated by cytotoxic T lymphocytes (CTLs), little is known about cell-cell interactions that regulate the CTL differentiation balance between effector and memory cells. Furthermore, cell-cell interactions that are required for peripheral tolerance of autoreactive B cells and CD8^{+} T cells are poorly understood.

This year, we published our finding about the role for S1PR2, the type-2 receptor for sphingosine-1-phosphate, in follicular helper T (Tfh) cell localization in germinal centers. Tfh cells are essential for initiation and maintenance of T-dependent B cell responses, particularly germinal center (GC) reactions, which lead to high affinity antibody production and humoral immune memory. A subpopulation of Tfh cells that has physical access to the GC is believed to be responsible for controlling GC reactions. However, little was known about mechanisms of Tfh cell access to GCs except for a partial dependence on the chemokine receptor CXCR5. Using the S1pr2-reporter mice and real time two-photon microscopy, we demonstrated that S1pr2 was expressed at various levels by Tfh cells and that Tfh cells with high S1pr2 expression are retained in the GC in an S1PR2-dependent manner. Furthermore, we showed that double deficiency of S1PR2 and CXCR5 in T cells severely impaired their localization to GCs and ability to support GC B cells. Thus, our study suggests that S1PR2 plays a cooperative role with CXCR5 in Tfh cell biology.

Recent Major Publications

Natsuaki Y, Egawa G, Nakamizo S, Ono S, Hanakawa S, Okada T, Kusuba N, Otsuka A, Kitoh A, Honda T, Nakajima S, Tsuchiya S, Sugimoto Y, Ishii KJ, Tsutsui H, Yagita H, Iwakura Y, Kubo M, Ng Lg, Hashimoto T, Fuentes J, Guttman-Yassky E, Miyachi Y, Kabashima K. Perivascular leukocyte clusters are essential for efficient activation of effector T cells in the skin. ***Nat Immunol*** 15, 1064–9 (2014)

Kawamoto S, Maruya M, Kato LM, Suda W, Atarashi K, Doi Y, Tsutsui Y, Qin H, Honda K, Okada T, Hattori M, Fagarasan S. Foxp3+ T cells regulate immunoglobulin a selection and facilitate diversification of bacterial species responsible for immune homeostasis. ***Immunity*** 41, 152–65 (2014)

Moriyama S, Takahashi N, Green JA, Hori S, Kubo M, Cyster JG, Okada T. Sphingosine-1-phosphate receptor 2 is critical for follicular helper T cell retention in germinal centers. ***J Exp Med*** 211, 1297–305 (2014)

Invited Presentations

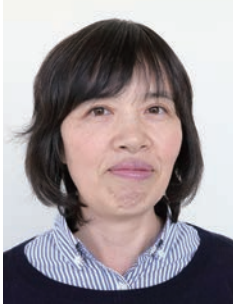
Okada T. Imaging of cellular dynamics during adaptive immune responses. International Symposium on Multi-dimensional Fluorescence Live Imaging of Cellular Functions and Molecular Activities, Kyoto, Japan. January, 2015.

Okada T. Cellular dynamics of adaptive immune responses in the lymph node. 44th Australasian Society for Immunology Annual Scientific Meeting, Wollongong, Australia. December, 2014.

Okada T. Imaging of anti-tumor immune response by cytotoxic T lymphocytes. The 73rd Annual Meeting of the Japanese Cancer Association, Yokohama, Japan. September, 2014.

Okada T. Imaging of adaptive immune responses in the lymph node. The 37th Naito Conference: Bioimaging-a paradigm shift for the life sciences, Hokkaido, Japan. July, 2014.

Okada T. Mechanism of follicular helper T cell localization in germinal centers. RIKEN IMS-JSI International Symposium on Immunology 2014, Yokohama, Japan. June, 2014.



Laboratory for Integrated Cellular Systems

Team Leader: Mariko Okada

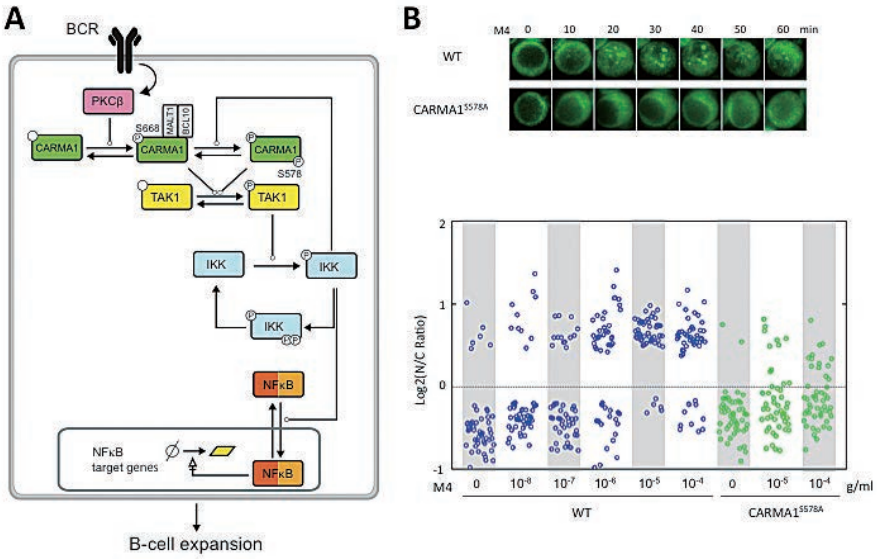


Figure: Modeling of Signal-transcription Network
(A) The NF-κB activation pathway in BCR signaling. The letter P in a circle indicates the phosphorylated state of the amino acid residue. (B) Single cell imaging analysis of GFP-tagged RelA nuclear translocation. (top) A representative time-course response in WT and CARMA-1^{S578A} cells after BCR stimulation with the M4 anti-IgM antibody. (bottom) M4 Dose response in WT (blue) and CARMA1^{S578A} (green) cells. Nuclear-cytoplasmic (N/C) fluorescence ratios at 45 min after stimulation showed that the WT cells were digitally activated in response to increased concentrations of the M4 antibody, while CARMA1^{S578A} cells instead showed a gradual increase.

The aims of the laboratory are to define the general regulatory rules in signal-transcription networks and to apply this knowledge of regulatory principles to the understanding of development of human diseases. Mammalian signal transduction pathways have developed to sense, sort and transfer a variety of extracellular information to transcription factors in the nucleus to regulate gene expression, thereby they link the genome and the environment. Interestingly, signaling pathways often control these processes in a nonlinear fashion and, in some cases, analogous graded doses of extracellular stimuli such as growth factors and antigens promote digital activation of transcription factors. Time and space-resolved context-dependent network topology plays an essential function to realize these types of responses. We developed several mathematical models of signal-transcription networks in immune cell development and cancer. In B cell signaling, based on quantitative experiments and mathematical modeling, we identified a positive feedback loop from IKK to TAK1 mediated by CARMA1 that induces switch activation of the NF-κB transcription factor. The feedback loop contributes to determining the threshold for NF-κB-mediated B cell proliferation, thereby serving as a mechanism for digital activation of B cells. Our studies suggest that cellular complexity might arise from combinatorial regulation of binary states of transcription factors. Moreover, to accomplish accurate prediction of combinatorial regulation of transcription factors in biological systems, we work together with our collaborators to develop computational algorithms that can be applied to the analysis of various Omics data.

Recent Major Publications

Nagashima T, Inoue N, Yumoto N, Saeki Y, Magi S, Volinsky N, Sorkin A, Kholodenko BN, Okada-Hatakeyama M. Feedforward regulation of mRNA stability by prolonged extracellular signal-regulated kinase activity. ***FEBS J*** 282, 613–29 (2015)

Shinohara H, Behar M, Inoue K, Hiroshima M, Yasuda,T, Nagashima T, Kimura S, Sanjo H, Maeda S, Yumoto N, Ki S, Akira S, Sako Y, Hoffmann A, Kurosaki T, Okada-Hatakeyama M. Positive feedback within a kinase signaling complex functions as a switch mechanism for NF-κB activation. ***Science*** 344, 760–4 (2014)

Kajiyama K, Okada-Hatakeyama M, Hayashizaki Y, Kawaji H, Suzuki H. Capturing drug responses by quantitative promoter activity profiling. ***CPT Pharmacometrics Syst Pharmacol*** 2, e77 (2013)

Invited Presentations

Okada-Hatakeyama M. Analog to Digital Conversion in Signal-Transcription Networks. The Third BMIRC International Symposium for Virtual Physiological Human, Izuka, Japan. March, 2015.

Okada-Hatakeyama M. Switch mechanism of biological network: implications for epigenetic regulation. RIKEN Epigenetics Project Meeting, Kobe, Japan. February, 2015.

Okada-Hatakeyama M. Omics cross-talk and cell regulation. The 87th Annual Meeting of the Japanese Biochemical Society, Kyoto, Japan. October, 2014.

Shinohara H. Swicth mechanism in B cell activation. IMSUT Seminar, Tokyo, Japan. June, 2014.

Laboratory for Molecular Live-Cell Quantification

Team Leader: Makio Tokunaga

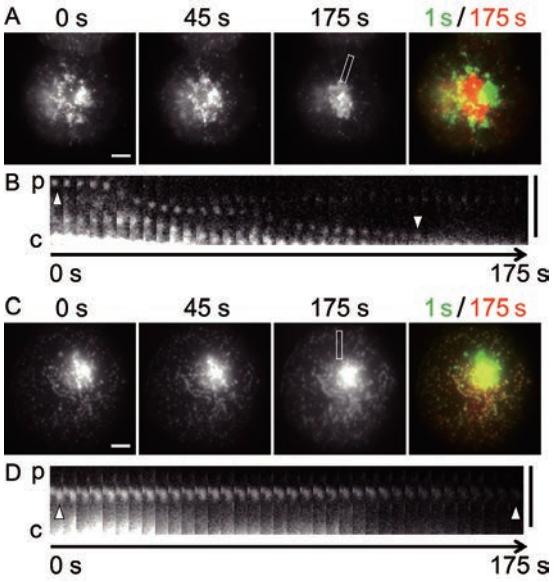


Figure: Facile Preparation of Glass-Supported Lipid Bilayers for Analyzing Molecular Dynamics
Time-lapse fluorescence images of Jurkat T cells expressing CD3ζ-TagRFP-T after stimulation on a planar bilayer (A, B) and on an antibody-coated glass surface (C, D). The images in the rectangular white boxes of (A and C) were clipped and rearranged in chronological order (B and D, respectively). Representative microclusters are indicated by arrowheads. p: periphery, c: center. Bars, 3 μm.

Based on emerging techniques in molecular imaging, our long-term goal is the understanding of transcriptional regulation related to cell signaling. To achieve this goal we quantify the interactions of signaling molecules and transcriptional regulators.

Development tools and methods for analyzing molecular dynamics

Many research programs focus on the molecular dynamics of living cells, an approach that requires cells to be adhered to a substrate while retaining the innate motility of their surface molecules. Lipid bilayer-based systems fulfill this requirement, although current methods are complicated and their utility is limited. We developed a simple and rapid method for reproducible preparation of homogeneous glass-supported lipid bilayers. Our method provides a facile means for bioimaging and analysis of molecular dynamics both *in vitro* and *in vivo*, especially in living cells.

Integrated quantitative analysis of molecular dynamics and interactions

Thanks to the development of better techniques for fluorescent labeling of bio-molecules in living cells, the target of imaging analysis is being expanded in its spatio-temporal resolution. For example, photo-activatable GFP variants enable labeling of a limited number of the proteins, an approach that yields super-resolution images of two different protein molecules. We are developing an integrated method for quantitative analysis of molecular dynamics and interactions, combining multiple methods of microscopy. Integrated use of multi-color single-molecule imaging, single molecule tracking, super-resolution microscopy, fluorescence recovery after photobleaching (FRAP) and/or fluorescence cross-correlation spectroscopy (FCCS) provide a means to obtain new view of spatio-temporal and kinetic parameters of molecular dynamics and interactions.

Recent Major Publications

Asakawa H, Yang HJ, Yamamoto TG, Ohtsuki C, Chikashige Y, Sakata-Sogawa K, Tokunaga M, Iwamoto M, Hiraoka Y, Haraguchi T. Characterization of nuclear pore complex components in fission yeast Schizosaccharomyces pombe. ***Nucleus*** 5, 149–62 (2014)

Stasevich TJ, Hayashi-Takanaka Y, Sato Y, Maehara K, Ohkawa Y, Sakata-Sogawa K, Tokunaga M, Nagase T, Nozaki N, McNally JG, Kimura H. Regulation of RNA polymerase II activation by histone acetylation in single living cells. ***Nature*** 516, 272–5 (2014)

Ito Y, Sakata-Sogawa K, Tokunaga M. A Facile Preparation of Glass-supported Lipid Bilayers for Analyzing Molecular Dynamics. ***Anal Sci*** 30, 1103–6 (2014), selected as Hot Article Award.

Invited Presentations

Sakata-Sogawa K, Ito Y, Fukagawa A, Tokunaga M. Integrated imaging approach to the study of dynamics of chromatin. The 52th Annual Meeting of the Biophysical Society of Japan: Symposium Session on "Studies of dynamic chromatin structure and function to understand fundamentals of life", Sapporo, Japan. September, 2014.

Laboratory for Metabolomics

Team Leader: Makoto Arita

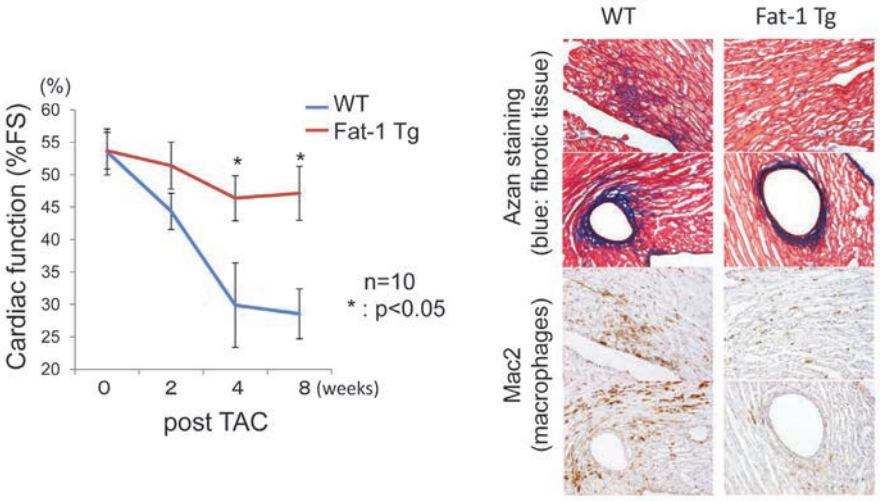


Figure: Preserved cardiac function and less cardiac fibrosis in omega-3 PUFA-enriched Fat-1 Tg mice under pressure overload
Mice were subjected to sustained pressure overload by transverse aortic constriction (TAC). Echocardiographic analysis revealed that the pressure overload-induced decline in percentage fractional shortening (%FS) observed in the WT mice was significantly alleviated in Fat-1 Tg mice (left). Pressure overload-induced perivascular and interstitial fibrosis and the accumulation of activated macrophages expressing Mac2 in the fibrotic tissue area were attenuated in Fat-1 Tg mice as compared to the WT mice (right). Azan staining (top) and immunohistochemistry for Mac2 (bottom) in ventricle sections from WT and Fat-1 Tg mice 4 weeks after TAC.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based lipidomics involves the simultaneous and quantitative analysis of metabolites in biological systems. When combined with proteomic, transcriptomic, and genomic profiles (multi-omics profiling), it can greatly assist in understanding the role of lipids and their metabolites in normal physiological and/or pathological conditions. This technology could potentially identify the metabolic fingerprint of a disease for clinical diagnosis and treatment. Moreover, identification of novel lipid metabolites with anti-inflammatory and tissue-protective actions could lead to the development of novel therapeutics for human disease when sustained inflammation is suspected as a key component of pathogenesis.

Dietary fatty acid balance is recognized as an important factor in immune regulation and disease control. In 2014, using a combination of genetic and lipidomic approachs, we discovered the molecular mechanisms underlying the anti-inflammatory and cardioprotective effects of omega-3 polyunsaturated fatty acids (PUFAs). Omega-3 PUFAs such as EPA and DHA are widely thought to be cardioprotective, although the direct evidence and mechanisms underlying such effects had been unclear. We demonstrated that Fat-1 transgenic (Fat-1 Tg) mice, a genetic model with elevated omega-3 PUFA levels in cells and tissues, were resistant to pressure overload-induced cardiac remodeling and heart failure. Bone marrow transplantation experiments revealed that Fat-1 Tg bone marrow cells, but not Fat-1 Tg cardiac cells, contributed to the cardioprotective effect. LC-MS/MS-based lipidomic analyses revealed significant enrichment of EPA and the EPA-metabolite 18-HEPE in Fat-1 Tg macrophages, and that the 18-HEPE-rich milieu in Fat-1 Tg heart was formed by bone marrow-derived Fat-1 Tg macrophages. *In vitro*, 18-HEPE inhibited pro-inflammatory activation of cardiac fibroblasts in culture, and *in vivo* administration of 18-HEPE conferred resistance to maladaptive cardiac remodeling. The use of 18-HEPE may become a novel therapeutic application for heart failure based on its anti-inflammatory and anti-fibrotic activities.

Recent Major Publications

Tani Y, Isobe Y, Imoto Y, Segi-Nishida E, Sugimoto Y, Arai H, Arita M. Eosinophils control the resolution of inflammation and draining lymph node hypertrophy through the proresolving mediators and CXCL13 pathway in mice. ***FASEB J*** 28, 4036–43 (2014)

Endo J, Sano M, Isobe Y, Fukuda K, Kang JX, Arai H, Arita M. 18-HEPE, an n-3 fatty acid metabolite released by macrophages, prevents pressure overload-induced maladaptive cardiac remodeling. ***J Exp Med*** 211, 1673–87 (2014)

Kubota T, Arita M, Isobe Y, Iwamoto R, Goto T, Yoshioka T, Urabe D, Inoue M, Arai H. Eicosapentaenoic acid is converted via ω-3 epoxygenation to the anti-inflammatory metabolite 12-hydroxy-17,18-epoxyeicosatetraenoic acid. ***FASEB J*** 28, 586–93 (2014)

Invited Presentations

Arita M. Metabolomics of n-3 PUFA and the regulation of inflammation. The 87th Annual Meeting of the Japanese Biochemical Society, Kyoto, Japan. October, 2014.

Arita M. Genetic and lipidomic approach for the anti-inflammatory properties of omega-3 fatty acid. 55th International Conference on the Bioscience of Lipids, Aberdeen, Scotland. June, 2014.

Arita M. Mediator lipidomics approach to understand the roles of fatty acid metabolism in controlling inflammation and tissue homeostasis, 18th Annual Meeting of Intestinal Microbiology, Tokyo, Japan. June, 2014.

Arita M. Lipid machinery involved in the resolution of inflammation. The 134th Annual Meeting of the Pharmaceutical Society of Japan, Kumamoto, Japan. March, 2014.

Arita M. Emerging roles of lipid mediators in controlling inflammation and resolution. Korea Society of Molecular and Cellular Biology, Division of Lipid Biology Symposium, Busan, Korea. February, 2014.

Core for Genomic Medicine

Photo: Twelve laboratories tackle the most advanced research
The Core for Genomic Medicine consists of four research groups and eight research teams. Each team is linked systematically with the others and works toward the implementation of personalized medicine.

The Core for Genomic Medicine is performing genomic research on human diseases, especially the common diseases. The aims of Core for Genomic Medicine are 1) to identify genetic variations related to disease susceptibility, disease outcome and drug responses (efficacy/adverse reaction), 2) to provide useful information about possible molecular targets for drug discovery, 3) to examine the interactions between genetic and environmental factors to understand the pathogenesis and the progression of diseases, and 4) finally to construct the evidence base for the implementation of personalized medicine.

To identify genetic variations related to disease susceptibility and drug responses, the Core for Genomic Medicine first showed the proof of concept of the genome-wide association study (GWAS) in 2002. To advance this strategy, the Core for Genomic Medicine has organized laboratories to facilitate comprehensive genomic research on common diseases. To produce comprehensive genomic information, the Laboratory for Genotyping Development is mainly working on large-scale SNP genotyping and genome sequencing for various diseases. The resulting huge amount of genomic variation data was mainly analyzed at the Laboratory for Statistical Analysis to extract significant genomic variations related to disease susceptibility and drug responses. These laboratories are in close communication with the research group of pharmacogenomics (Laboratory for Pharmacogenomics and Laboratory for International Alliance on Genomic Research), laboratories for disease-causing mechanisms (Laboratory for Cardiovascular Diseases, Autoimmune Diseases, Digestive Diseases, Bone and Joint Diseases, Endocrinology, Metabolism and Kidney Diseases, and Respiratory and Allergic Diseases) and many other collaborators worldwide for further analyses. In addition to this strategy, the Laboratory for Genome Sequencing Analysis is mainly working on whole genome sequencing of cancer genomes to clarify the pathogenesis of carcinogenesis.

Laboratory for Genotyping Development

Group Director: Michiaki Kubo

Photo: Illumina Human Genotyping Array system (left) and HiSeq 2500 System (right)

During the era of the SNP Research Center (FY2000-2007), our team established a high-throughput SNP genotyping system using a combined method of multiplex-PCR and the Invader assay. Using this system, our team contributed to the establishment of the JSNP database (http://snp.ims.u-tokyo.ac.jp/index_ja.html) and to the success of the International HapMap Project Phase 1 (http://hapmap.ncbi.nlm.nih.gov/). From FY2003, our team has been working as the main genomic research facility for the BioBank Japan project and has generated a large amount of SNP genotyping data for association studies of common diseases. In addition, since 2008 we have been performing genotyping of samples collected by NIH for a pharmacogenetic study, Pharmacogenomics Research Network (PGRN), under the PGRN-RIKEN CGM Global Alliance (http://bts.ucsf.edu/pgrn-cgm/). Moreover, we developed a new genotyping method to detect copy number variation (RETINA) that was published in Human Mutation (29, 182–9, 2008). Using RETINA, we developed a new genotyping method for the *CYP2D6* gene, which has many functional variations combined with copy number variations. From 2013, our team introduced next-generation sequencers to further analyze the association of genomic variations with various phenotypes, including disease susceptibility and drug responses. We also developed a rapid SNP genotyping system for clinical research and are performing several clinical intervention studies based on the genotype information in the Genome-guided drug Treatment Optimization Program (G-TOP) funded by MEXT. Our hope is to implement Genomic Medicine that will optimize medical care and health by use of genomic information.

Recent Major Publications

Cai Q, Zhang B, Sung H, Low SK, Kweon SS, Lu W, Shi J, Long J, Wen W, Choi JY, Noh DY, Shen CY, Matsuo K, Teo SH, Kim MK, Khoo US, Iwasaki M, Hartman M, Takahashi A, Ashikawa K, Matsuda K, Shin MH, Park MH, Zheng Y, Xiang YB, Ji BT, Park SK, Wu PE, Hsiung CN, Ito H et al. Genome-wide association analysis in East Asians identifies breast cancer susceptibility loci at 1q32.1, 5q14.3 and 15q26.1. ***Nat Genet*** 46, 886–90 (2014)

Zhang B, Jia WH, Matsuda K, Kweon SS, Matsuo K, Xiang YB, Shin A, Jee SH, Kim DH, Cai Q, Long J, Shi J, Wen W, Yang G, Zhang Y, Li C, Li B, Guo Y, Ren Z, Ji BT, Pan ZZ, Takahashi A, Shin MH, Matsuda F, Gao YT, Oh JH, Kim S, Ahn YO; Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO), Chan AT, Chang-Claude J et al. Large-scale genetic study in East Asians identifies six new loci associated with colorectal cancer risk. ***Nat Genet*** 46, 533–42 (2014)

Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A, Yoshida S, Graham RR, Manoharan A, Ortmann W, Bhangale T, Denny JC, Carroll RJ, Eyler AE, Greenberg JD, Kremer JM, Pappas DA, Jiang L, Yin J, Ye L, Su DF, Yang J, Xie G, Keystone E, Westra HJ, Esko T, Metspalu A et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. ***Nature*** 506, 376–81 (2014)

Invited Presentations

Kubo, M. The impact of genome-wide association study on clinical medicine: future perspective. Mahidol University Research Expo 2014, Nakhon Pathom, Thailand. December, 2014.

Kubo M. From genomic research to genomic medicine and prevention. The 59th Annual Meeting of the Japan Society of Human Genetics, Tokyo, Japan. November, 2014.

Kubo M. The BioBank Japan Project and the implementation of Personalized Medicine. 41st IMSUT Founding Commemorative Symposium "New Frontiers in Human Genome Science," Tokyo, Japan. May, 2014.

Kubo M. Pharmacogenetics in the BioBank Japan project. The 3rd Meeting of South East Asian Pharmacogenomics Research Network (SEAPharm), Jakarta, Indonesia. Apri, 2014.

Laboratory for Genome Sequencing Analysis

Team Leader: Hidewaki Nakagawa

Recent Major Publications

Fujimoto A, Furuta M, Shiraishi Y, Gotoh K, Kawakami Y, Arihiro K, Nakamura T, Ueno M, Ariizumi S, Hai Nguyen H, Shigemizu D, Abe T, Boroevich KA, Nakano K, Sasaki A, Kitada R, Maejima K, Yamamoto Y, Tanaka H, Shibuya T, Shibata T, Ojima H, Shimada K, Hayami S, Shigekawa Y, Aikata H, Ohdan H, Marubashi S, Yamada T, Kubo K, Hirano S, Ishikawa O, Yamamoto M, Yamaue H, Chayama K, Miyano S, Tsunoda T, Nakagawa H. Whole-genome mutational landscape of liver cancers displaying biliary phenotype reveals hepatitis impact and molecular diversity. ***Nat Commun*** 6, 6120 (2015)

Shiraishi Y, Fujimoto A, Furuta M, Tanaka H, Chiba K, Boroevich KA, Abe T, Kawakami Y, Ueno M, Gotoh K, Ariizumi S, Shibuya T, Nakano K, Sasaki A, Maejima K, Kitada R, Hayami S, Shigekawa Y, Marubashi S, Yamada T, Kubo M, Ishikawa O, Aikata H, Arihiro K, Ohdan H, Yamamoto M, Yamaue H, Chayama K, Tsunoda T, Miyano S, Nakagawa H. Integrated analysis of whole genome and transcriptome sequencing reveals diverse transcriptomic aberrations driven by somatic genomic changes in liver cancers. ***PLoS One*** 9, e114263 (2014)

Al Olama AA, Kote-Jarai Z, Berndt SI, Conti DV, Schumacher F, Han Y, Benlloch S, …Breast and Prostate Cancer Cohort Consortium (BPC3), PRACTICAL (Prostate Cancer Association Group to Investigate Cancer-Associated Alterations in the Genome) Consortium, COGS (Collaborative Oncological Gene-environment Study) Consortium, GAME-ON/ELLIPSE Consortium, Cook MB, Nakagawa H, Wiklund F, Kraft P, Chanock SJ, Henderson BE, Easton DF, Eeles RA, Haiman CA. A meta-analysis of 87,040 individuals identifies 23 new susceptibility loci for prostate cancer. ***Nat Genet*** 46, 1103–9 (2014)

Invited Presentations

Nakagawa H. Decoding liver cancer genome by whole genome sequencing. 14th Japanese-German Cancer Workshop, Berlin, Germany. November, 2014.

Nakagawa H. SNP-based risk estimation for prostate cancer and personalized prostate cancer screening. The 52nd Annual Meeting of Japan Society of Clinical Oncology (JSCO2014), Yokohama, Japan. August, 2014.

Nakagawa H. Decoding liver cancer genome toward personalized medicine. 11th International Conference of the Asian Clinical Oncology Society in conjunction with the 19th Taiwan Joint Cancer Conference, Taipei, Taiwan. May, 2014.

Nakagawa H. Risk estimation of prostate cancer by genomic information and pseronalzied PSA screening. The 102nd Annual Meeting of the Japanese Urological Association, Kobe, Japan. April, 2014.

Nakagawa H. Whole genome pictures of live cancers and personalized medicine. Major Symposium of American Association for Cancer Research (AACR) Annual Meeting 2014, San Diego, USA. April, 2014.

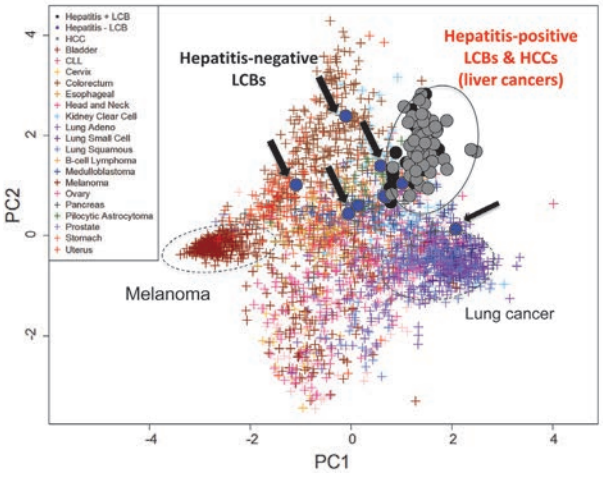


Figure: Genome-wide substitution patterns in hepatocellular (HCC) and biliary (LCB) phenotype liver cancers
Principal component analysis (PCA) of the whole-genome substitution patterns of the 30 LCBs the 60 HCCs and other types of cancers. Hepatitis-positive LCBs (black dots) overlap the HCC cluster (gray). LCBs developed in livers without hepatitis (blue dots) diverged from others. Hepatitis-positive liver cancers (black and gray dots) are tightly clustered, as are melanomas and lung cancers, indicating that chronic hepatitis can strongly impact the somatic mutation signature.

We have been analyzing 300 whole genome sequencing (WGS) and RNA-seq datasets for liver cancer as a Japanese ICGC project, collaborating with the National Cancer Research Center and the University of Tokyo. In this study, we have identified several mutational clusters in non-coding regions and rearrangements, in addition to several coding driver mutations, in 300 liver cancer genomes and are now analyzing their biological and functional significance in cancer development. These datasets were registered to ICGC and for the PanCancer WGS project (PCAWG) in ICGC/TCGA, which is contributing to 12.5% of the WGS dataset worldwide (300/2400). As one of PIs of PCAWG, we are involved with some collaborative projects, including driver gene analysis, mutational signatures, immune-genomics, and mitochondrial genomics, and have established several analytic pipelines for these missions in the cancer WGS project. Together with the University of Tokyo, we organized the Tokyo "cloud" data center for PCAWG, where six world-class genome centers play technically central roles in the analysis of ~5000 WGS datasets for the PCAWG vertical network. We systematically performed integrated analysis of WGS and RNA-seq of 22 HBV-related liver cancers, an effort that could improve the interpretation of the consequences of non-coding mutations and rearrangements (PLoS One, 2014). We also analyzed 30 WGS of biliary phenotype liver cancers and demonstrated the strong impact of chronic hepatitis on the mutational landscape in liver cancer (Nat Commun, 2015). As of 2014, we completed WGS/exome of 120 bile duct cancers, 23 IBD-related or microsatellite instability (MSI) MSI colorectal cancers, and 20 chemo-radiation sensitive/resistant esophageal cancers, and WGS of 10 Vietnam families, who were affected with dioxin exposure and congenital diseases. We participated in global meta-analysis of prostate cancer GWAS and identified 23 new loci for prostate cancer susceptibility, which have now reached 100 loci (Nat Genet, 2014), and were involved with meta-analysis of Asian prostate cancer GWAS.

Laboratory for Medical Science Mathematics

Group Director: Tatsuhiko Tsunoda

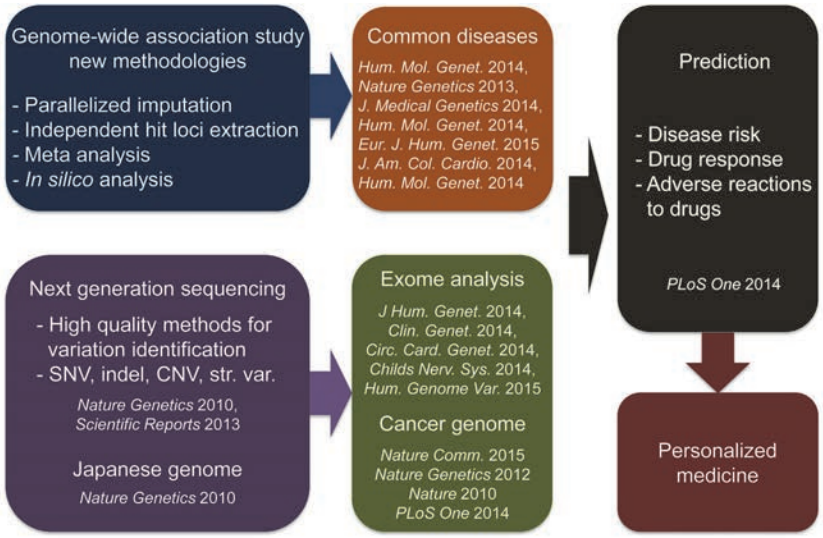


Figure: Development and application of our new analysis methods

Our research centers around the development of mathematical methods to enhance understanding of genetic variation and its relationship to human disease. First, to enhance ongoing GWAS analyses, we developed parallelized imputation pipelines to infer data for ungenotyped SNPs, developed methods to extract independent loci from the high-density data, and applied these methods to a number of GWAS. This resulted in the identification of several genes related to diseases/physiological traits, e.g. type 2 diabetes (Hum Mol Genet 23, 239–46, 2014), idiopathic scoliosis (Nat Genet 45, 676–9, 2013; J Med Genet 51, 401–6, 2014) and height (Hum Mol Genet 24, 1791–800, 2015). Also, we contributed to GWAS signal pathway analysis (Eur J Hum Genet, 23, 374–80, 2015), and conducted meta-analyses of atrial fibrillation and BMI GWASes (Circulation 130, 1225–35, 2014; J Am Coll Cardiol 63, 1200–10, 2014; Hum Mol Genet 23, 5492–504, 2014). Using GWAS data, the Bayesian method, and a machine learning technique, we constructed an improved risk prediction system for type 2 diabetes (PLoS One 9, e92549, 2014). Recently, massively parallel sequencing technology has allowed the creation of comprehensive catalogs of genetic variation. However, to overcome relatively high sequencing error rates, more sophisticated analysis methods are required. Expanding on our analysis of the first reported whole genome sequence of a Japanese individual (Nat Genet 42, 931–6, 2010), we developed methods for detecting SNVs and short indels in whole genome and exome sequencing data (Sci Rep 3, 2161, 2013). As an International Cancer Genome Consortium (ICGC) member (Nature 464, 993–8, 2010), we constructed an analytical pipeline based on this method to detect somatic alterations. Using this pipeline, we reported our analyses of hepatocellular carcinoma (HCC) genomes (Nat Genet 44, 760–4, 2012), liver cancers with biliary phenotype (Nat Commun, 6, 6120, 2015), and transcriptomes associated with somatic mutations of HCC (PLoS One 9, e114263, 2014). Lastly, we applied our pipeline to various exome analyses, which resulted in the identification of several disease causing genes (J Hum Genet 59, 639–41, 2014; Clin Genet, doi: 10.1111/cge.12492; Circ Cardiovasc Genet 7, 466–74, 2014; Childs Nervous System, 31, 465–71, 2015; Human Genome Variation, 2, 15007, 2015).

Recent Major Publications

Fujimoto A, Furuta M, Shiraishi Y, Nguyen HH, Shigemizu D, Gotoh K, Kawakami Y, Nakamura T, Ueno M, Ariizumi S, Shibata T, Abe T, Boroevich KA, Nakano K, Sasaki A, Kitada R, Maejima K, Yamamoto Y, Tanaka H, Shibuya T, Ojima H, Shimada K, Hayami S, Shigekawa Y, Aikata H, Arihiro K, Ohdan H, Marubashi S, Yamada T, Ishikawa O, Kubo M, Hirano S, Yamamoto M, Yamaue H, Chayama K, Miyano S, Tsunoda T*, Nakagawa H* (*: co-last). Whole-genome mutational landscape of liver cancers displaying biliary phenotype reveals hepatitis impact and molecular diversity. ***Nat Commun*** 6, 6120 (2015)

He M+, Xu M+, Zhang B+, Liang J+, Chen P+, Lee JY+, Johnson TA+, Li H+, …, Kubo M, …, Okada Y, …, Takeuchi F, Tanaka T, …, Shen H*, Teo YY*, Mo Z*, Wong TY*, Lin X*, Mohlke KL*, Ning G*, Tsunoda T*, Han BG*, Shu XO*, Tai ES*, Wu T*, Qi L* (+: co-first, *: co-last). Meta-analysis of genome-wide association studies of adult height in East Asians identifies 17 novel loci. ***Hum Mol Genet*** 24, 1791–800 (2015)

Shigemizu D, Abe T, Morizono T, Johnson TA, Boroevich KA, Hirakawa Y, Ninomiya T, Kiyohara Y, Kubo M, Nakamura Y, Maeda S, Tsunoda T. The construction of risk prediction models using GWAS data and its application to a type 2 diabetes prospective cohort. ***PLoS One*** 9, e92549 (2014)

Invited Presentations

Tsunoda T. Exploring etiologies, sub-classification, and risk prediction of diseases based on big-data analysis of clinical and whole omics data in medicine. CREST Symposium on Big Data Application, Tokyo, Japan. March, 2015.

Tsunoda T. Medical science mathematics for cancer genome analysis. The 73rd Annual Meeting of the Japanese Cancer Association, Yokohama, Japan. September, 2014.

Shigemizu D. Analysis for disease-causing mutations using whole-exome sequencing data. International Symposium on Bioinformatics and its Application, Tokyo, Japan. September, 2014.

Tsunoda T. Whole genome big data analysis for personalized medicine. Fujitsu Knowledge, Inovation, and Drug Consoritum, Tokyo, Japan. April, 2014.

Tsunoda T. Whole genome big data analysis for complex diseases. 3rd Global COE Workshop between BGI and University of Tokyo - Advances in Medical Genomics, Tokyo, Japan. March, 2014.

Laboratory for Statistical Analysis

Team Leader: Atsushi Takahashi

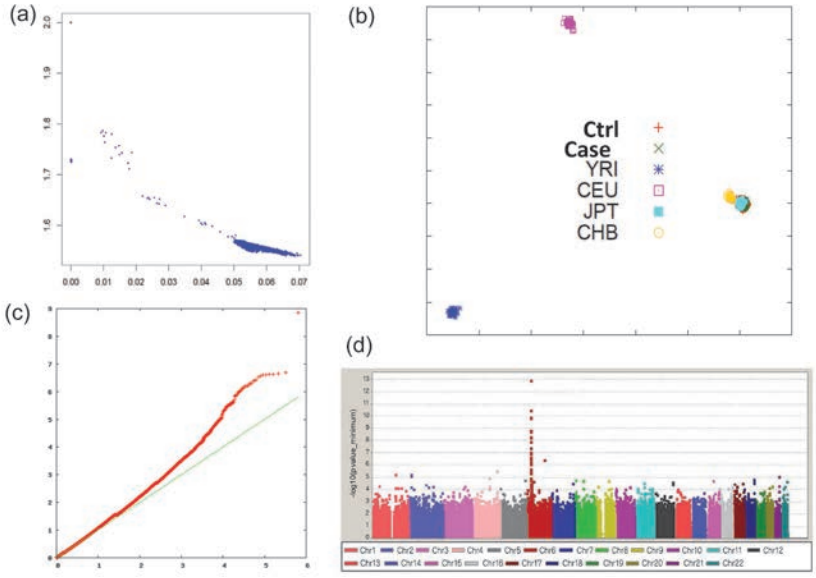


Figure: Representative results of GWAS Analysis
(a) relatedness beween subjects (b) population stratification (c) q-q plot (d) manhattan plot

The mission of our laboratory is to clarify the mechanisms of human diseases and traits from the viewpoint of statistics and informatics. Recent technology developments have enabled us to investigate human variations over the entire genome. Massive amounts of genomic data are now available, and we try to identify the genes associated with diseases/traits by performing analysis of these data.

Our center searches for genes associated with diseases and drug reactions. Our laboratory is in charge of performing GWAS and selecting candidate SNPs associated with diseases. We are performing many quality controls on the SNP data based on statistical genetics and statistics to obtain interpretable results. Then our laboratory is performing case-control association studies or quantitative analyses.

The Biobank Japan project has collected samples from approximately 200,000 patients with 47 diseases, along with clinical and phenotypic information. IMS has genotyped an enormous number of the SNPs of individuals in the Biobank Japan. By using these data, we try to find new loci associated with diseases by GWAS. We also have constructed and developed GWAS systems to perform the GWAS in a shorter time. Individual genotype data have been accumulated and there are very large amounts of genomic data. Therefore, we have constructed a data system able to manage all these data. We are in charge of GWAS and statistical analysis at IMS and collaborate in many projects/consortiums to find novel loci associated with diseases, drug reactions and traits.

Our center has started whole genome and whole exome sequencing as part of the Tailor-made Medical Treatment Program. Our laboratory is attempting to establish a very accurate system to perform analysis of data produced by the next generation sequencer (NGS). Based on our system, we plan to find loci associated with diseases.

Recent Major Publications

Nakajima M, Takahashi A, Tsuji T, Karasugi T, Baba H, Uchida K, Kawabata S, Okawa A, Shindo S, Takeuchi K, Taniguchi Y, Maeda S, Kashii M, Seichi A, Nakajima H, Kawaguchi Y, Fujibayashi S, Takahata M, Tanaka T, Watanabe K, Kida K, Kanchiku T, Ito Z, Mori K, Kaito T, Kobayashi S, Yamada K, Takahashi M, Chiba K, Matsumoto M, Furukawa K, Kubo M, Toyama Y, Ikegawa S. A genome-wide association study identifies susceptibility loci for ossification of the posterior longitudinal ligament of the spine. ***Nat Genet*** 46, 1012–6 (2014)

Low SK, Takahashi A, Mushiroda T, Kubo M. Genome-wide association study: a useful tool to identify common genetic variants associated with drug toxicity and efficacy in cancer pharmacogenomics. ***Clin Cancer Res*** 20, 2541–52 (2014)

Low SK, Takahashi A, Ashikawa K, Inazawa J, Miki Y, Kubo M, Nakamura Y, Katagiri T. Genome-wide association study of breast cancer in the Japanese population. ***PLoS One*** 8, e76463 (2013)

Invited Presentations

Takahashi A. Japanese population structure evaluated by single nucleotide polymorphism. Research Meeting in National Institute of Genetics, Mishima, Japan. December, 2014.

Takahashi A. Large scale genome analysis and clinical application. The 24 Annual Meeting of The Japanese Society of Clinical Neuropsychopharmacology / The 44 Annual Meeting of the Japanese Society of Neuropsychopharmacology, Nagoya, Japan. November, 2014.

Low SK. The role of common genetic variations in complex diseases and pharmacogenomics studies. Education Seminar in University of Sydney, Sydney, Australia. October, 2014.

Low SK. Genome-wide association studies identify genetic variants associated with drug efficacy and drug-induced toxicity. The 73rd Annual Meeting of Japanese Cancer Association, Yokohama, Japan. September, 2014.

Low SK. 1. Path to personalized medicine: role of genetic contributions to pharmacogenomics studies, 2. Utilization of genome-wide association studies (GWAS) to decipher genetic architecture of complex diseases. Education Seminar in University of Malaya, Kuala Lumpur, Malaysia. June, 2014.

Laboratory for Pharmacogenomics

Group Director: Taisei Mushiroda

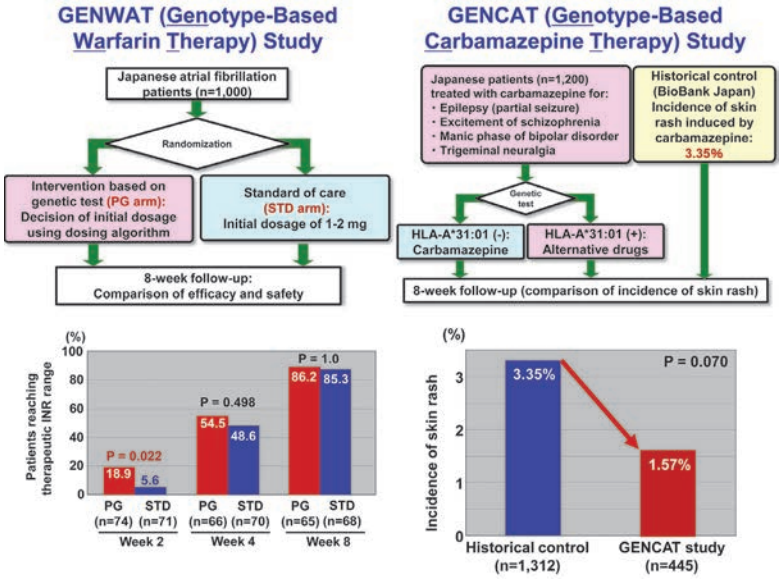


Figure: Study designs and interim analyses of prospective clinical trials, GENWAT and GENCAT studies
The GENWAT Study is being conducted in order to validate a warfarin dosing algorithm using genetic information, SNPs of *CYP2C9* and *VKORC1*. The percentage of patients reaching therapeutic international normalized ratio (INR) range was 18.9% in the genotype-guided group (PG arm) as compared with 5.6% in the control group (STD arm) (P = 0.022). In GENCAT study, we validate a prediction system for risk of carbamazepine-induced skin rash by a genetic test of *HLA-A*31:01*. The advanced genetic test reduced the prevalence of carbamazepine-induced skin rash from 3.35% in the historical control group to 1.57% in the intervention group, indicating the medical utility of the genetic test using *HLA-A*31:01*.

Adverse drug reactions (ADRs) are often unpredictable, owing to the fact that responses to drugs vary among different individuals. However, it is believed that applying knowledge of pharmacogenomics (PGx) in clinical treatment can help to improve predictions of drug efficacy and/or toxicity, leading to appropriate therapeutic regimens for individual patients and to contribute to improvements in medical care. In fact, the U.S. Food and Drug Administration (FDA) recommended genotyping of polymorphisms in drug-metabolizing enzymes and human leukocyte antigen (HLA) genes prior to drug administration to help avoid severe ADRs for several drugs, such as irinotecan, atomoxetine, carbamazepine and abacavir. In attempts to identify genomic biomarkers that predict efficacy or risk of ADRs for various drugs, such as neutropenia and leucopenia induced by cancer chemotherapeutic agents and skin rash induced by anti-epileptics, we conduct genome-wide association studies (GWAS) using single-nucleotide polymorphisms (SNPs), which are the most abundant polymorphisms in the human genome. To date, we have identified several "probable valid" genomic biomarkers (*HLA-B*35:05/CCHCR1* for nevirapine-induced skin rash, *VKORC1/CYP2C9* for warfarin maintenance dosage, *HLA-A*31:01* for carbamazepine-induced skin rash, and *CYP2D6* for tamoxifen efficacy) that will be useful for PGx-guided drug therapy. In order to establish PGx-based individualization of drug therapy, advantages of the genomic biomarkers should be demonstrated. Thus, we have conducted and are conducting prospective clinical trials that can evaluate the medical utility and cost-effectiveness of genetic testing. If physicians can predict in advance which patients are more susceptible to ADRs, they could use alternative drugs or take particular care during the course of treatment, e.g., to prevent the nevirapine -induced skin rash at an early stage, leading to safe and patient-friendly personalized treatment.

Recent Major Publications

Chin YM, Mushiroda T, Takahashi A, Kubo M, Krishnan G, Yap LF, Teo SH, Lim PV, Yap YY, Pua KC, Kamatani N, Nakamura Y, Sam CK, Khoo AS; Malaysian NPC Study Group, Ng CC. HLA-A SNPs and amino acid variants are associated with nasopharyngeal carcinoma in Malaysian Chinese. ***Int J Cancer*** 136, 678–87 (2015)

Province MA, Goetz MP, Brauch H, Flockhart DA, Hebert JM, Whaley R, Suman VJ, Schroth W, Winter S, Zembutsu H, Mushiroda T, Newman WG, Lee MT, Ambrosone CB, Beckmann MW, Choi JY, Dieudonné AS, Fasching PA, Ferraldeschi R, Gong L, Haschke-Becher E, Howell A, Jordan LB, Hamann U, Kiyotani K, Krippl P, Lambrechts D, Latif A, Langsenlehner U, Lorizio W et al. CYP2D6 genotype and adjuvant tamoxifen: meta-analysis of heterogeneous study populations. ***Clin Pharmacol Ther*** 95, 216–27 (2014)

Perera MA, Cavallari LH, Limdi NA, Gamazon ER, Konkashbaev A, Daneshjou R, Pluzhnikov A, Crawford DC, Wang J, Liu N, Tatonetti N, Bourgeois S, Takahashi H, Bradford Y, Burkley BM, Desnick RJ, Halperin JL, Khalifa SI, Langaee TY, Lubitz SA, Nutescu EA, Oetjens M, Shahin MH, Patel SR, Sagreiya H, Tector M, Weck KE, Rieder MJ, Scott SA, Wu AH et al. Genetic variants associated with warfarin dose in African-American individuals: a genome-wide association study. ***Lancet*** 382, 790–6 (2013)

Invited Presentations

Mushiroda, T. Identification of genomic biomarkers associated with cutaneous adverse drug reactions and validation of medical utility of genetic testing. The 14th Annual Meeting of the East Asian Union of Human Genetics Societies, Tokyo, Japan. November, 2014.

Mushiroda, T. Pharmacogenomics-based individualization of drug therapy. The 22nd Annual Medical Sciences Conference, Bangkok, Thailand. June, 2014.

Laboratory for International Alliance on Genomic Research

Team Leader: Ming Ta Michael Lee

Recent Major Publications

Caudle KE, Rettie AE, Whirl-Carrillo M, Smith LH, Mintzer S, Lee MT, Klein TE, Callaghan JT. Clinical pharmacogenetics implementation consortium guidelines for CYP2C9 and HLA-B genotype and phenytoin dosing. ***Clin Pharmacol*** Ther 96, 542–8 (2014)

Song IW, Li WR, Chen LY, Shen LF, Liu KM, Yen JJ, Chen YJ, Chen YJ, Kraus VB, Wu JY, Lee MT*, Chen YT* (*: co-corresponding). Palmitoyl acyltransferase, Zdhhc13, facilitates bone mass acquisition by regulating postnatal epiphyseal development and endochondral ossification: a mouse model. ***PLoS One*** 9, e92194 (2014)

Chen CH, Lee CS, Lee MT, Ouyang WC, Chen CC, Chong MY, Wu JY, Tan HK, Lee YC, Chuo LJ, Chiu NY, Tsang HY, Chang TJ, Lung FW, Chiu CH, Chang CH, Chen YS, Hou YM, Chen CC, Lai TJ, Tung CL, Chen CY, Lane HY, Su TP, Feng J, Lin JJ, Chang CJ, Teng PR, Liu CY, Chen CK, Liu IC, Chen JJ, Lu T, Fan CC, Wu CK, Li CF, Wang KH, Wu LS, Peng HL, Chang CP, Lu LS, Chen YT, Cheng AT. Variant GADL1 and Response to Lithium Therapy in Bipolar I Disorder. ***N Engl J Med*** 370, 119–28 (2014)

Invited Presentations

Lee MT. International Clopidogrel Pharmacogenetics Consortium Genome Wide Association Study. 14th Joint Meeting of Coronary Revascularization, Busan, Korea. December, 2014.

Lee MT. Direct expression and methylation assessment of articular cartilage and subchondral bone on human osteoarthritic knee. The 59th Annual Meeting of the Japan Society of Human Genetics, Tokyo, Japan. November, 2014.

Lee MT. Pharmacogenetic study of drug induced severe cutaneous disorders. Genomics of Rare Diseases Serbordisinn & 2014 Golden Helix Symposium, Belgrade, Serbia. October, 2014.

Lee MT. Potential application of pharmacogenomics in psychiatric diseases. 3rd Meeting of South East Asian Pharmacogenomics Research Network, Jakarta, Indonesia. April, 2014.

Lee MT. Moving Towards Translation: Using Pharmacogenetics as an example for personalized medicine. The Fourth Military Medical University, Shaanxi, China. February, 2014.

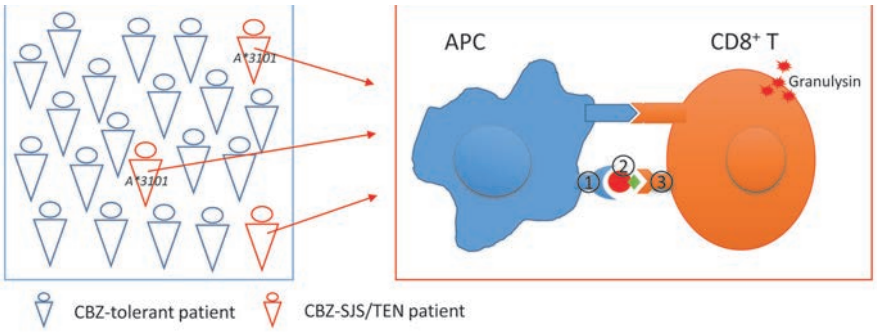


Figure: Using Immune Diversity to Elucidate the Underlying Mechanism of CBZ-induced SJS/TEN in the Japanese Population

1. HLA allele diversity in the population: Carriers with the HLA-A*3101 allele have a much higher risk to develop CBZ-SJS/TEN.
2. Peptide diversity: Drug modification causes a shifted repertoire of new peptides that become "non-self" antigens.
3. TCR diversity: Drug-specific TCRs may have a different TCR repertoire. This can explain why not all the HLA-A*3101 carriers develop CBZ-SJS/TEN.

Granulysin is an effector molecule used by CD8 T cells to kill target cells.

The main aim of our laboratory is to identify genetic associations with diseases and with drug induced adverse events or drug efficacy. It is hoped that the discoveries from our research will identify useful biomarkers that could be used to predict drug-induced adverse events, guide drug use and could also be used in disease prediction/diagnosis. Another main focus of this laboratory is to promote collaborations both within Japan and internationally. We have set up collaborations/consortia in Asia (Southeast Asian Pharmacogenetics Consortium, SEA-Pharm) and Europe (Genomic Medicine Alliance) to foster collaboration with RIKEN and among the participating research groups. We also actively recruit young scientists from overseas to work at RIKEN and carry out research in SNP-based approaches, statistical analysis and biological analysis. Our goal is to establish an international genetic research network that will make the Center for Integrative Medical Sciences a world leader in personalized medicine. Currently, our group's main focus is on Pharmacogenetics (PGx) and the genetic study of complex diseases. For PGx studies, we aim to establish a functional analysis platform that not only allows us to study the interactions between HLA and drugs, but also can be used to confirm the findings from genetic analysis for severe ADRs, many of which involve the HLA molecules. In addition, we will use genome-wide association study to identify genetic variants associated with 1. Anti-infectious drug-induced liver injuries. 2. Phenytoin and co-trimoxazole induced Stevens-Johnson Syndrome (SJS) and Toxic epidermal necrolysis. 3. Adverse reactions to non-steroidal anti-inflammatory drugs. For complex diseases, we aim to identify genetic variants associated with Hippocampal Sclerosis in Thais and are performing an epigenetic and biomarker study of osteoarthritis (OA).

Laboratory for Cardiovascular Diseases

Group Director: Toshihiro Tanaka

Table1: Meta-analyses of SNP associations with AF by origin of the study population

SNP	Chromosome	AF Risk Allele	Closest Gene	Relative Location	Original GWAS Data Set[4] RAF	RR (95% CI)	P	Replication RAF	RR (95% CI)	P	Overall Meta-Analysis RAF	RR (95% CI)	P
Europeans													
rs12415501	10q24	T	*NEURL*	Intronic	0.16	1.15 (1.10–1.22)	9.0×10^{-8}	0.16	1.22 (1.14–1.29)	6.0×10^{-10}*	0.16	1.18 (1.13–1.23)	6.5×10^{-16}*
rs10507248	12q24	T	*TBX5*	Intronic	0.73	1.13 (1.08–1.18)	8.5×10^{-8}	0.73	1.11 (1.05–1.17)	0.0001*	0.73	1.12 (1.08–1.16)	5.7×10^{-11}*
rs4642101	3p25	G	*CAND2*	Intronic	0.65	1.11 (1.06–1.15)	4.2×10^{-6}	0.65	1.09 (1.04–1.15)	0.0006*	0.65	1.10 (1.06–1.14)	9.8×10^{-9}*
rs13216675	6q22	T	*GJA1*	Intergenic	0.69	1.10 (1.05–1.15)	5.0×10^{-5}	0.68	1.10 (1.05–1.16)	0.0001*	0.69	1.10 (1.06–1.14)	2.2×10^{-8}*
Japanese													
rs6584555	10q24	C	*NEURL*	Intronic	0.12	1.33 (1.14–1.55)	2.8×10^{-4}	0.12	1.32 (1.25–1.39)	1.6×10^{-22}*	0.12	1.32 (1.26–1.39)	2.0×10^{-25}*
rs6490029	12q24	A	*CUX2*	Intronic	0.65	1.22 (1.09–1.37)	6.3×10^{-4}	0.64	1.11 (1.07–1.16)	5.0×10^{-7}*	0.64	1.12 (1.08–1.16)	3.9×10^{-9}*

Since cardiovascular diseases cause more than 15% of the deaths in the Japanese population and represent more than 20% of the total medical expenses in Japan, it is socially important to discover the mechanisms of these disorders. We have been among the first to reveal genetic background effects in myocardial infarction (MI), atrial fibrillation (AF), Kawasaki disease (KD), and peripheral artery disease (PAD) by comprehensive genetic analyses of the Japanese population followed by functional *in vitro* analyses. Our ultimate goal is to provide novel diagnostic/therapeutic approaches to such patients. To this end, we are extending our research area from genetics to molecular biology, including *in vivo* analyses of genetically engineered mice. Also, we are making efforts to develop a new drug for MI that would elute from a stent, a powerful tool for coronary angioplasty.

Recent Major Publications

Sinner MF, Tucker NR, Lunetta KL, Ozaki K, Smith JG, Trompet S, Bis JC, Lin H, Chung MK, Nielsen JB, Lubitz SA, Krijthe BP, Magnani JW, Ye J, Gollob MH, Tsunoda T, Müller-Nurasyid M, Lichtner P, Peters A, Dolmatova E, Kubo M, Smith JD, Psaty BM, Smith NL, Jukema JW, Chasman DI, Albert CM, Ebana Y, Furukawa T, MacFarlane P, Harris TB, Darbar D, Dörr M, Holst AG, Svendsen JH, Hofman A, Uitterlinden A, Gudnason V, Isobe M, Malik R, Dichgans M, Rosand J, Van Wagoner DR; METASTROKE Consortium; AFGen Consortium, Benjamin EJ, Milan DJ, Melander O, Heckbert S, Ford I, Liu Y, Barnard J, Olesen MS, Stricker BH, Tanaka T, Kääb S, Ellinor PT. Integrating genetic, transcriptional, and functional analyses to identify five novel genes for atrial fibrillation. ***Circulation*** 130, 1225–35 (2014)

Lubitz SA, Lunetta KL, Lin H, Arking DE, Trompet S, Li G, Krijthe BP, Chasman DI, Barnard J, Kleber ME, Dörr M, Ozaki K, Smith AV, Müller M, Walter S, Agarwal SK, Bis JC, Brody JA, Chen LY, Everett BM, Ford I, Franco OH, Harris TB, Hofman A, Kääb S, Mahida S, Kathiresan S, Kubo M, Launer LJ, MacFarlane PW, Magnani JW, McKnight B, McManus DD, Peters A, Psaty BM, Rose LM, Rotter JI, Silbernagel G, Smith JD, Sotoodehnia N, Stott DJ, Taylor K, Tomaschitz A, Tsunoda T, Uitterlinden AG, Van Wagoner DR, Völker U, Völzke H, Murabito JM, Sinner MF, Gudnason V, Felix SB, März W, Chung M, Albert CM, Stricker BH, Tanaka T, Heckbert SR, Jukema JW, Alonso A, Benjamin EJ, Ellinor PT. Novel genetic markers associate with atrial fibrillation risk in Europeans and Japanese. ***J Am Coll Cardiol*** 63, 1200–10 (2014)

Invited Presentations

Tanaka T. Genome and medicine. Forum on Cardiovascular Diseases, Osaka, Japan. June, 2014.

Onouchi Y. Genome wide association studies and Kawasaki disease. Pediatric Academic Societies and Asian Society for Pediatric Research Joint Meeting, Vancouver, Canada. May, 2014.

Tanaka T. Genetic epidemiology. 11th CEM Forum, Kyoto, Japan. April, 2014.

Onouchi Y. Pathophysiological basis for genetic research in Kawasaki disease. American College of Cardiology 63rd Annual Scientific Session, Washington DC, USA. March, 2014.

Laboratory for Autoimmune Diseases

Team Leader: Kazuhiko Yamamoto

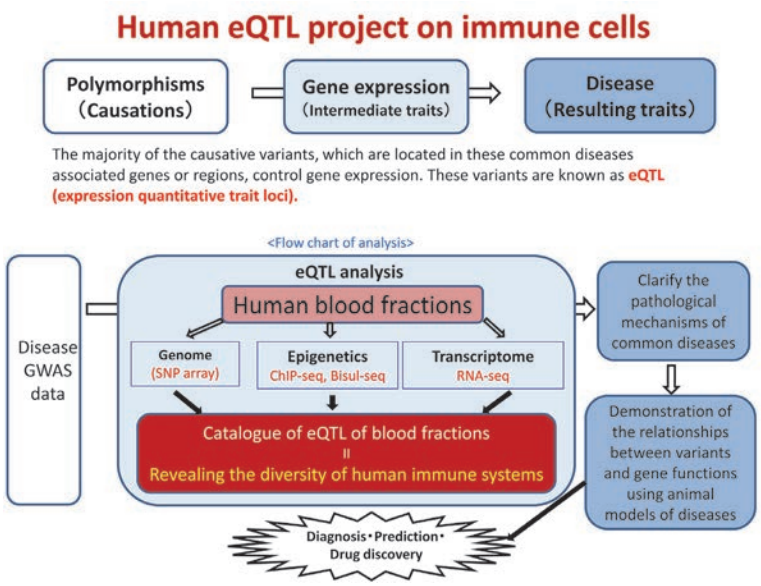


Figure: Understanding autoimmune diseases through eQTL studies
We focus on the expression of genes as an intermediate trait to understand the mechanisms of autoimmune diseases. We are performing eQTL studies for each immune cell type by using next generation sequencing technologies such as RNA-seq. By combining the data from GWAS and eQTL studies, we will unravel the mechanisms of disease.

Most autoimmune diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus, are multifactorial, involving both genetic and environmental factors. The aim of our laboratory is to elucidate the etiology of these autoimmune diseases by identifying their genetic aspects. RA is one of the most common autoimmune diseases with an inflammatory arthritis component. While the *HLA-DRB1* gene polymorphism is the major determinant of RA susceptibility, several groups worldwide including our own have performed genome-wide association studies (GWAS) to find non-HLA risk loci. However, each individual GWAS lacked adequate statistical power and thus a substantial proportion of risk loci remained undiscovered. In world-wide collaborations, we performed multi-ethnic meta-analyses of GWAS and have found more than 100 risk loci for RA so far.

As GWAS could only indicate the presence of disease-associated variants in the loci, we further investigated these candidate loci to seek disease-causal variants and elucidate their biological relevance to RA. Previous expression quantitative trait loci (eQTL) studies that examined association between genetic variants and gene expression levels have suggested that the majority of autoimmune loci are eQTLs, where disease causing variants affect expression of the responsible genes. Therefore, we believe it is essential to focus on "gene expression" as an intermediate trait to dissect the etiology of "disease onset". We are currently undertaking genome and transcriptome analysis of each immune cell type from Japanese individuals to establish eQTL catalogues of human immune cells. In addition, as the precise function of the responsible genes in vivo has been not yet clarified for most of the candidate loci, we are investigating the function of these genes including *PADI4*, *CD244 FCRL3*, and *RTKN2* by using gene-targeting mice and disease-model mice. Furthermore, to facilitate the development of new therapies, we are performing a high-throughput screening of novel therapeutic compounds targeting RA risk genes in a collaboration with the RIKEN Program for Drug Discovery and Medical Technology Platforms.

Recent Major Publications

Yamamoto K, Okada Y, Suzuki A, Kochi Y. Genetics of rheumatoid arthritis in Asia—present and future. ***Nat Rev Rheumatol***, in press

Kochi Y, Suzuki A, Yamamoto K. Genetic basis of rheumatoid arthritis: a current review. ***Biochem Biophys Res Commun*** 452, 254–62 (2014)

Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A, Yoshida S, Graham RR, Manoharan A, Ortmann W, Bhangale T, Denny JC, Carroll RJ, Eyler AE, Greenberg JD, Kremer JM, Pappas DA, Jiang L, Yin J, Ye L, Su DF, Yang J, Xie G, Keystone E, Westra HJ, Esko T, Metspalu A et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. ***Nature*** 506, 376–81 (2014)

Invited Presentations

Yamamoto K. Current and future genetics of rheumatoid arthritis in Asia. The 59th Annual Meeting of the Japan Society of Human Genetics, Tokyo, Japan. Tokyo, Japan. November, 2014.

Yamamoto K. A brief history of GWAS and next generation genetics of RA. Rheumatoid arthritis genetics meeting, Boston, U.S.A. November, 2014.

Yamamoto K. Genetics and epigenetics in autoimmune diseases. 6th International Forum on Rheumatoid Arthritis, Beijing, China. September, 2014.

Yamamoto K. TGF-β3-producing $CD4^+CD25^-LAG3^+$ regulatory T cells control B cell responses. Cold Spring Habor Asia Conferences "Frontiers of Immunology in Health and Diseases," Suzhou, China. September, 2014.

Yamamoto K. T cell targeted therapies in autoimmune diseases. Advanced Target Therapies, Athens, Greece. March, 2014.

Laboratory for Digestive Diseases

Team Leader: Kazuaki Chayama

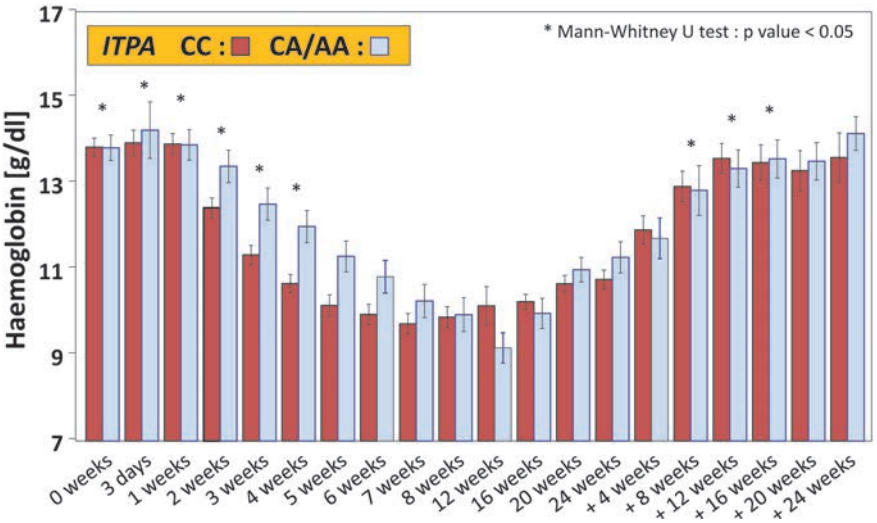


Figure: Haemoglobin levels by *ITPA* rs1127354 genotype during 24 weeks of telaprevir/ribavirin/peg-interferon therapy and 24 weeks of follow-up
Decline of haemoglobin was significantly faster, and ribavirin was more extensively reduced in patients with *ITPA* SNP rs1127354 genotype CC than CA/AA. Post marketing-phase triple therapy resulted in a high sustained viral response rate in spite of extensive ribavirin dose reduction in a diverse patient population, indicating the importance of treatment continuation and appropriate management of adverse events.

Our laboratory has mainly used GWAS to investigate the host genetic factors underlying various diseases and responsiveness to therapy, such as chronic HBV (Nat Genet, 2009; Hum Mol Genet, 2011) and HCV infection (PLoS One, 2013), HCV-induced liver cirrhosis (J Hepatol, 2013) and cancer (Nat Genet, 2011), response to interferon therapy (J Gen Virol, 2011), and ribavirin-induced anemia (Gastroenterology, 2010). We have also participated in whole-genome sequencing analysis of liver cancers (Nat Genet, 2012).

We have now intensively investigated and verified how to apply this genetic information to clinical practice (Hepatology, 2014; Antivir Ther, 2014; Hepatol Res, 2014; J Gastroenterol, in press). We estimated haplotype structure of the *IFNL4/IL-28B* locus, consisting of 3 SNPs, and found that 2 of them may have better predictive impact on response to PEG-interferon/ribavirin therapy (J Gen Virol, 2014). We found ribavirin dose reduction during telaprevir/ribavirin/PEG-interferon triple therapy overcomes the effect of the *ITPA* gene polymorphism on ribavirin-induced anemia (J Viral Hepat, in press).

We have studied not only the human but also the hepatitis virus genome to predict and improve treatment response using *in vitro* and *in vivo* infection models (Gut, 2013; Antimicrob Agents Chemother, 2014). We studied drug resistance by using HCV-infected chimeric mice treated with direct-acting antiviral agents (DAAs) and concluded that sequential use of DAAs should be avoided to prevent emergence of multidrug-resistant strains (Am J Gastroenterol, 2013).

In addition, we are currently investigating the host immune response to viral infection to elucidate the mechanism of hepatitis (Hepatology, 2011&2012; Biochem Biophys Res Commun, 2013), since our GWAS suggested the importance of the immune system for the etiology of hepatitis B and C.

Our ultimate goals are (1) establishment of personalized medicine for various liver diseases and (2) contribution to development of new therapies, diagnostic methods, and prophylactic approaches to various liver diseases.

Recent Major Publications

Ochi H, Miki D, Hayes CN, Abe H, Hayashida Y, Kubo M, Chayama K. IFNL4/IL-28B haplotype structure and its impact on susceptibility to hepatitis C virus and treatment response in the Japanese population. ***J Gen Virol*** 95, 1297–1306 (2014)

Miki D, Ochi H, Takahashi A, Hayes CN, Urabe Y, Abe H, Kawaoka T, Tsuge M, Hiraga N, Imamura M, Kawakami Y, Aikata H, Takahashi S, Akuta N, Suzuki F, Ikeda K, Kumada H, Karino Y, Toyota J, Tsunoda T, Kubo M, Kamatani N, Nakamura Y, Chayama K. HLA-DQB1*03 confers susceptibility to chronic hepatitis C in Japanese: a genome-wide association study. ***PLoS One*** 8, e84226 (2013).

Abe Y, Aly HH, Hiraga N, Imamura M, Wakita T, Shimotohno K, Chayama K, Hijikata M. Thromboxane A2 synthase inhibitors prevent production of infectious hepatitis C virus in mice with humanized livers. ***Gastroenterology*** 145, 658–67.e11 (2013).

Laboratory for Bone and Joint Diseases

Team Leader: Shiro Ikegawa

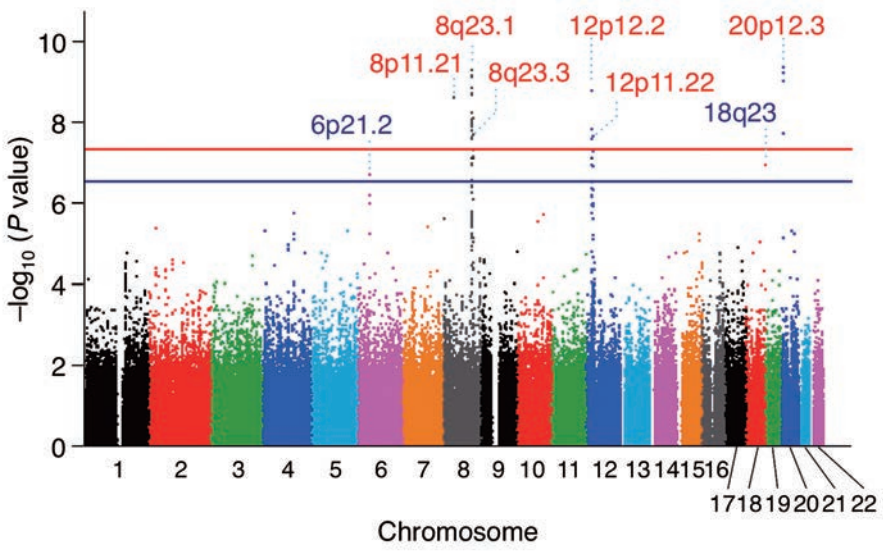


Figure: Genome-wide association study (GWAS) identified susceptibility loci for ossification of the posterior longitudinal ligament of the spine
Manhattan plot showing the –log10 P value from the GWAS. The values were plotted against their respective positions on the autosomal chromosomes. The red line represents the genome-wide significance threshold ($P = 5 \times 10^{-8}$). The blue line represents the threshold ($P = 5 \times 10^{-7}$) for selecting SNPs for the replication study.

Bone and joint diseases are serious problems throughout the world. We are working on identification of new susceptibility genes for many common bone and joint diseases, including osteoarthritis, osteoporosis, adolescent idiopathic scoliosis, lumbar disc herniation and ossification of the posterior longitudinal ligament of the spine (OPLL). This year, we succeeded in identification of new susceptibility genes for OPLL.

OPLL is one of the most common spinal disorders among the elderly and causes myelopathy and radiculopathy due to compression of the spinal cord and nerves by ectopic ossification of the ligament. More than 1–2% of Japanese are suffering from this intractable disease. To identify susceptibility genes for OPLL, we performed a genome-wide association study (GWAS), the first in the world for this disease. Through the GWAS in ~8,000 Japanese individuals followed by a replication study using an additional ~7,000 Japanese individuals, we identified six susceptibility loci for OPLL with genome-wide significance of the association: 20p12.3 (rs2423294: $P = 1.10 \times 10^{-13}$), 8q23.1 (rs374810: $P = 1.88 \times 10^{-13}$), 12p11.22 (rs1979679: $P = 4.34 \times 10^{-12}$), 12p12.2 (rs11045000: $P = 2.95 \times 10^{-11}$), 8q23.3 (rs13279799: $P = 1.28 \times 10^{-10}$) and 6p21.1 (rs927485: $P = 9.40 \times 10^{-9}$).

Analyses of gene expression in and around the loci using *in silico* and *in vitro* approaches suggested that several genes in the loci are involved in OPLL etiology through membranous and/or endochondral ossification processes. Our results bring new insight into the etiology of OPLL as well as mechanisms of ectopic ossification.

Recent Major Publications

Nakajima M, Takahashi A, Tsuji T, Karasugi T, Baba H, Uchida K, Kawabata S, Okawa A, Shindo S, Takeuchi K, Taniguchi Y, Maeda S, Kashii M, Seichi A, Nakajima H, Kawaguchi Y, Fujibayashi S, Takahata M, Tanaka T, Watanabe K, Kida K, Kanchiku T, Ito Z, Mori K, Kaito T, Kobayashi S, Yamada K, Takahashi M, Chiba K, Matsumoto M, Furukawa KI, Kubo M, Toyama Y; Genetic Study Group of Investigation Committee on Ossification of the Spinal Ligaments, Ikegawa S. A genome-wide association study identifies susceptibility loci for ossification of the posterior longitudinal ligament of the spine. ***Nat Genet*** 46, 1012–6 (2014)

Yamashita A, Morioka M, Kishi H, Kimura T, Yahara Y, Okada M, Fujita K, Sawai H, Ikegawa S, Tsumaki N. Statin treatment rescues FGFR3 skeletal dysplasia phenotypes. ***Nature*** 513, 507–11 (2014)

Tsurusaki Y, Koshimizu E, Ohashi H, Phadke S, Kou I, Shiina M, Suzuki T, Okamoto N, Imamura S, Yamashita M, Watanabe S, Yoshiura K, Kodera H, Miyatake S, Nakashima M, Saitsu H, Ogata K, Ikegawa S, Miyake N, Matsumoto N. De novo SOX11 mutations cause Coffin-Siris syndrome. ***Nat Commun*** 5, 4011 (2014)

Invited Presentations

Ikegawa S. Genomic study of common diseases. 1st Chinese Osteoarthritis Annual Congress, Nanjing, China. October, 2014.

Ikegawa S. Genomic study of skeletal diseases. 2nd Asia-Pacific Bone & Mineral Research Meeting, Seoul, Korea. May, 2014.

Ikegawa S. Genomic studies of bone and joint diseases: past, present and future. 1st Karolinska Workshop on Skeletal Dysplasia, Stockholm, Sweden. March, 2014.

Ikegawa S. Translational genomics in bone diseases. Invited lecture, The Master Program for Clinical Pharmacogenomics and Pharmacoproteomics, Taipei Medical University, Taipei, Taiwan. January, 2014.

Ikegawa S. Genomic study of common diseases: Road from genome to personalized medicine. Invited lecture, Taipei Medical University Hospital, Taipei, Taiwan. January, 2014.

Laboratory for Endocrinology, Metabolism and Kidney Diseases

Team Leader: Shiro Maeda

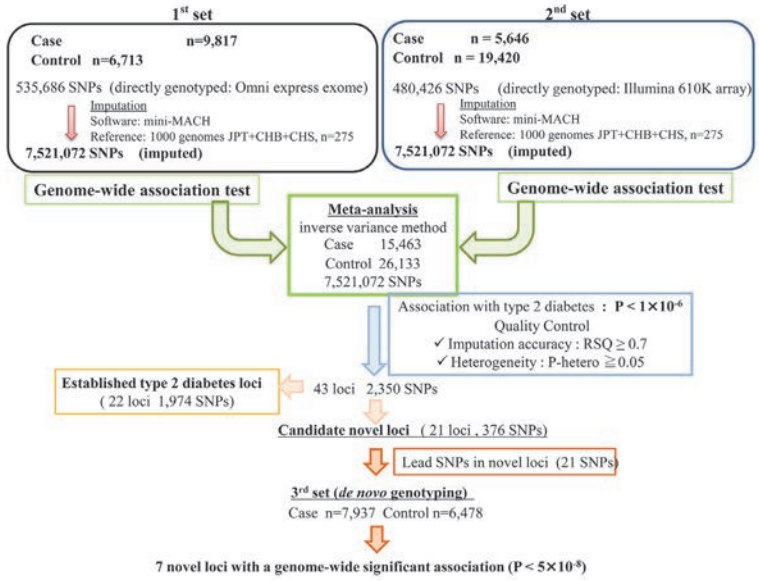


Figure: A meta-analysis of Japanese GWAS identified 7 new loci for type 2 diabetes

Research activities for type 2 diabetes:

We expanded our Japanese genome-wide association study (GWAS) for type 2 diabetes by increasing the number of examined SNPs (6,209,637 SNPs) and the number of participants (26,805 Japanese individuals, 5,976 patients with type 2 diabetes and 20,829 controls). After combining the results of candidate loci in the discovery stage and those in a follow-up case-control study (total number of participants, 30,392 type 2 diabetes cases and 34,814 controls) we identified three new loci for type 2 diabetes in the Japanese, *MIR129-LEP*, *GPSM1* and *SLC16A13* (Hara K *et al. Hum Mol Genet*, 2014). The associations of these 3 loci with type 2 diabetes were not replicated in European populations, but the *SLC16A13* locus was shown to be associated with type 2 diabetes in a Mexican population (Williams AL *et al. Nature*, 2014).

We further performed an independent Japanese GWAS for type 2 diabetes, in which ~7.5 million SNPs data obtained from genotype imputation performed by mini-MACH using directly genotyped data and reference data in the 1000 genomes (JPT+CHB+CHS, n = 275) were analyzed for 9,817 cases and 6,763 controls. Then these results were combined with GWAS data in the already published Japanese population described above. Obtained candidate SNP loci were further evaluated in an independent Japanese case-control study and, after integration of all results, we have identified 7 novel loci for type 2 diabetes in the Japanese (Fig.).

Simultaneously, in an international collaborative effort to identify common susceptibility loci for type 2 diabetes, we have been participating in a Trans-ethnic type 2 diabetes meta-analysis consortium; this analysis has identified 7 additional susceptibility loci for type 2 diabetes.

Other research activities:

We have been performing GWAS for diabetic nephropathy and diabetic retinopathy. We have also reported results of a comprehensive search for causal mutations in PKD1 and PKD2 in Japanese subjects with autosomal dominant polycystic kidney disease.

Recent Major Publications

Kurashige M, Hanaoka K, Imamura M, Udagawa T, Kawaguchi Y, Hasegawa T, Hosoya T, Yokoo T, Maeda S. A comprehensive search for mutations in the PKD1 and PKD2 in Japanese subjects with autosomal dominant polycystic kidney disease. ***Clin Genet*** 87, 266–72 (2015)

DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium; Asian Genetic Epidemiology Network Type 2 Diabetes (AGEN-T2D) Consortium; South Asian Type 2 Diabetes (SAT2D) Consortium; Mexican American Type 2 Diabetes (MAT2D) Consortium; Type 2 Diabetes Genetic Exploration by Next-generation sequencing in multi-Ethnic Samples (T2D-GENES) Consortium. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. ***Nat Genet*** 46, 234–44 (2014)

Hara K, Fujita H, Johnson TA, Yamauchi T, Yasuda K, Horikoshi M, Peng C, Hu C, Ma RC, Imamura M, Iwata M, Tsunoda T, Morizono T, Shojima N, So WY, Leung TF, Kwan P, Zhang R, Wang J, Yu W, Maegawa H, Hirose H; DIAGRAM consortium, Kaku K, Ito C, Watada H, Tanaka Y, Tobe K, Kashiwagi A, Kawamori R, Jia W, Chan JC, Teo YY, Shyong TE, Kamatani N, Kubo M, Maeda S, Kadowaki T. Genome-Wide Association Study Identifies Three Novel Loci for Type 2 Diabetes. ***Hum Mol Genet*** 23, 239–46 (2014)

Laboratory for Respiratory and Allergic Diseases

Team Leader: Mayumi Tamari

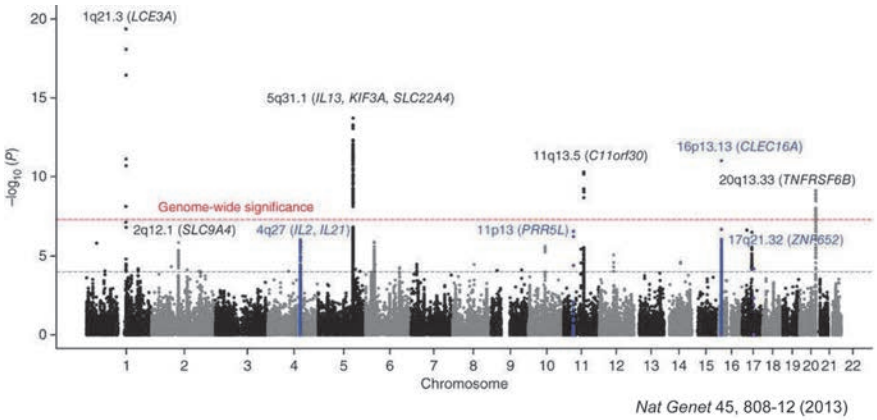


Figure: Manhattan plot of the Immunochip analysis for atopic dermatitis
We conducted an Immunochip analysis and validation studies. A total of four new atopic dermatitis susceptibility loci were identified with genome-wide significance: *IL2-IL21*, *PRR5L*, *CLEC16A* and *ZNF652*.

Recent Major Publications

Park HW, Dahlin A, Tse S, Duan QL, Schuemann B, Martinez FD, Peters SP, Szefler SJ, Lima JJ, Kubo M, Tamari M, Tantisira KG. Genetic predictors associated with improvement of asthma symptoms in response to inhaled corticosteroids. ***J Allergy Clin Immunol*** 133, 664–9 (2014)

Ellinghaus D, Baurecht H, Esparza-Gordillo J, Rodríguez E, Matanovic A, Marenholz I, Hübner N, Schaarschmidt H, Novak N, Michel S, Maintz L, Werfel T, Meyer-Hoffert U, Hotze M, Prokisch H, Heim K, Herder C, Hirota T, Tamari M, Kubo M, Takahashi A, Nakamura Y, Tsoi LC, Stuart P, Elder JT, Sun L, Zuo X, Yang S, Zhang X, Hoffmann P, Nöthen MM, Fölster-Holst R, Winkelmann J, Illig T, Boehm BO, Duerr RH, Büning C, Brand S, Glas J, McAleer MA, Fahy CM, Kabesch M, Brown S, McLean WH, Irvine AD, Schreiber S, Lee YA, Franke A, Weidinger S. High-density genotyping study identifies four new susceptibility loci for atopic dermatitis. ***Nat Genet*** 45, 808–12 (2013)

Tomita K, Sakashita M, Hirota T, Tanaka S, Masuyama K, Yamada T, Fujieda S, Miyatake A, Hizawa N, Kubo M, Nakamura Y, Tamari M. Variants in the 17q21 asthma susceptibility locus are associated with allergic rhinitis in the Japanese population. ***Allergy*** 68, 92–100 (2013)

Invited Presentations

Tamari M. Genetic mechanisms of allergic diseases. The 3rd One Airway One disease in Osaka, Osaka, Japan. November, 2014.

Tamari M. Genome-wide association studies of allergic diseases. The 24th Congress of Interasma Japan/ North Asia, Nagoya. Japan. July, 2014.

Tamari M. Mechanisms of allergic diseases –cross talk between genes and environment-. The 31th Annual Meeting of the Japanese Society of Pediatric Intractable Asthma and Allergic Diseases, Nagoya, Japan. June, 2014.

Tamari M. Genome-wide association study of atopic dermatitis. The 79th Annual Meeting of the Japanese Society of Interferon and Cytokine Research, Sapporo, Japan. June, 2014.

Hirota T. Genetic studies for allergy susceptibility genes. The 26th Spring Meeting of Japanese Society of Allergology, Kyoto, Japan. May. 2014.

The aim of our project is to improve our understanding of the pathophysiology of human respiratory and allergic diseases, which are caused by a combination of genetic and environmental factors.

We participate in the Global Alliance for Pharmacogenomics. We have conducted genome-wide association studies (GWASs) and identified a locus that is associated with improvement of asthma symptoms in response to inhaled corticosteroids. The related SNP lies in the intronic region of the *FBXL7* gene and is associated with decreased expression of this gene in immortalized B cells derived from patients with asthma (J Allergy Clin Immunol, 2014).

A number of GWASs have been conducted for allergic diseases and several susceptibility loci have been identified. We reported an immunochip analysis for atopic dermatitis, which revealed four new susceptibility loci, 4q27 (*IL-2-IL21*), 11p13 (*PRR5L*), 16p13.13 (*CLEC16A-DEXI*) and 17q21.32 (*ZNF652*) (Nat Genet, 2013). Candidate genes identified by the previous GWASs and immunochip analysis suggest roles for barrier functions, innate-adaptive immunity, IL-1 family signaling, regulatory T cells and the vitamin D pathway in the pathogenesis of allergic diseases. To investigate whether polymorphisms identified by these genetic studies could affect the susceptibility to and clinical phenotypes of diseases, we will conduct functional analyses and serological studies.

The first GWAS of asthma in the European population identified a locus on chromosome 17q21.1. Subsequent replication studies have identified genetic variants on the 17q21.1 locus associated with asthma in different ethnic populations. We reported that genetic variants in the 17q21.1 locus were associated with allergic rhinitis and were strongly correlated in *cis* with transcript levels of *ORMDL3* in Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines in a Japanese population (Allergy, 2013).

We will conduct further cross-disciplinary studies combining genetics, immunology and clinical epidemiology for translation of our research into clinical practice.

Program for Medical Innovations

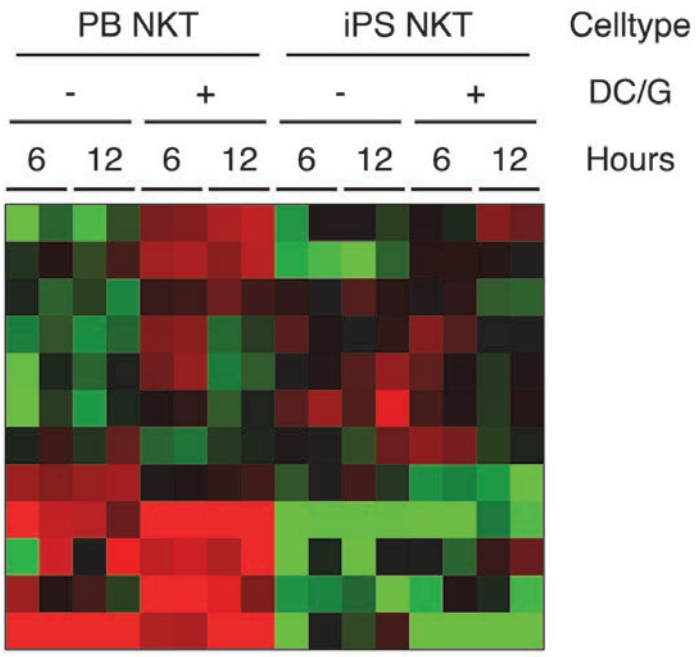


Figure: Differences in gene expression between peripheral blood and iPSC-derived human NKT cells.
Human NKT cells were generated from iPSC in OP9/dll-1 cultures. The iPS-derived and peripheral blood NKT cells were cultured for the indicated times with or without dendritic cells loaded with α-GalCer (DC/G). RNA sequence analysis was performed and the differences between expressed genes are depicted.

Six original projects for clinical applications have been performed in 2014: 1) A biochemical drug using a PEGylated Cryj-1/2 fusion recombinant protein for Cedar Pollinosis has been developed by the Torii pharmaceutical company and IMS and introduced to the Japanese market. 2) A chemical compound recently developed using the α-galactosylceramide (α-GalCer) analog, RCAI-X, selectively induces apoptosis of IgE but not IgG B cells and preferentially suppresses IgE production. It therefore potentially could be applied to any type of allergic disorder, such as pollinosis, food allergy, as well as allergic asthma. 3) NKT cell-targeted therapy for head and neck tumors has been done in collaboration with Chiba University, and was authorized by the Japanese government as Advanced Medical Treatment B in 2013. 4) The artificial adjuvant vector cell as an anti-tumor vaccine project has been accepted by the translational research network program and developed. This vaccine can be dosed with tumor antigen mRNA together with α-GalCer, so that it activates both innate and acquired protective immunity and also induces long-term memory. 5) The human iPS project for clinical use of *in vitro* generated NKT cells has been accepted as a Center for Clinical Application Research (Type B) in the Research Center Network for Realization of Regenerative Medicine, Japan in 2013. 6) A humanized mouse model for *MLL* gene-rearranged leukemia was established. Also, certain genes were identified that are differentially expressed between normal stem cells and leukemic stem cells.

Laboratory for Immune Regulation

Group Director: Masaru Taniguchi

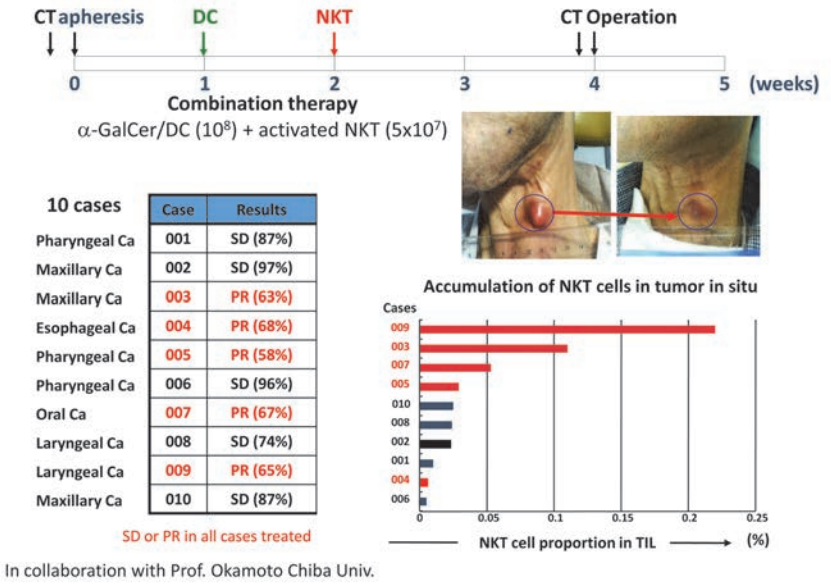


Figure: Phase II Clinical Trial of NKT cell–targeted therapy for head and neck tumors
The clinical study was carried out in collaboration with Prof. Okamoto, Chiba University Hospital. All 10 cases treated with the combination of α-GalCer/DCs and activated NKT cells showed dramatic clinical efficacy (Stable disease (SD) or partial remission (PR). Also note the significant correlation between clinical efficacy (PR in red, SD in black) and the number of NKT cells infiltrating the tumor *in situ*.

Identification of novel NKT cell development pathway: We identified an NKT cell subset developed at the thymic DN stage before selection by CD1d. The DN NKT cells seem different from NKT cells developing at the DP stage as judged by the following evidence: 1) RORγt, which is essential for generation and proliferation of NKT cells at the DP stage, is not necessary for DN NKT cell development, 2) both in-frame and out-of-frame Vα14Jα18 sequences are detected in DN fractions. Our findings reveal a novel NKT cell developmental pathway that is different from the DP pathway.

Cell intrinsic requirement for the transcription factor Maf in the development of the IL-17 producing NKT cell subset: To clarify the role of Maf, a master regulator of IL-4 expression, in NKT cells, we used Maf-deficient mice created by irradiation mixed fetal liver chimera. Maf was indispensable for the development of NKT17 cells in a cell intrinsic manner, but not for Th1 and Th2 type NKT cells. Therefore, our findings reveal a novel role for Maf in NKT17 cell differentiation.

Clinical trials of NKT-targeted therapy for lung cancer after surgery and for head and neck tumors: We are currently collaborating with the National Hospital Organization to carry out a randomized Phase II clinical trial on stage IIA-IIIA lung cancer after surgical tumor resection, which was approved as the advanced medical care assessment system B in 2014.

iPS-derived NKT cells: The project on the development of human iPS from NKT cells and iPS-derived NKT cells has been accepted as the Center for Clinical Application Research (Type B) in the Research Center Network for Realization of Regenerative Medicine, Japan (headed by Dr. Koseki). We successfully developed human iPS-derived NKT cells with desired functions and established the method for functional NKT cells suitable for anti-cancer therapy.

Recent Major Publications

Tarumoto N, Kinjo Y, Kitano N, Sasai D, Ueno K, Okawara A, Izawa Y, Shinozaki M, Watarai H, Taniguchi M, Takeyama H, Maesaki S, Shibuya K, Miyazaki Y. Exacerbation of invasive Candida albicans infection by commensal bacteria or a glycolipid through IFN-γ produced in part by iNKT cells. ***J Infect Dis*** 209, 799–810 (2014)

Shimizu K, Sato Y, Shinga J, Watanabe T, Endo T, Asakura M, Yamasaki S, Kawahara K, Kinjo Y, Kitamura H, Watarai H, Ishii Y, Tsuji M, Taniguchi M, Ohara O, Fujii S. KLRG+ invariant natural killer T cells are long-lived effectors. ***Proc Natl Acad Sci U S A*** 111, 12474–9 (2014)

Ren Y, Dashtsoodol N, Watarai H, Koseki H, Quan C, Taniguchi M. Generation of induced pluripotent stem cell-derived mice by reprogramming of a mature NKT cell. ***Int Immunol*** 26, 551–61 (2014)

Invited Presentations

Taniguchi M. Development of a drug suppressing total IgE. The 51st Annual Meeting of Japanese Society of Pediatric Allergy and Clinical Immunology, Yokkaichi, Japan. November, 2014.

Taniguchi M. Discovery of NKT cells and their clinical application. 16th JSI Immunology Summer School , Shodoshima Island, Japan. July, 2014.

Taniguchi M. Novel anti-cancer immunotherapy targeted on NKT cells. The 10th Hiroshima Liver Project Research Center Symposium, Hiroshima, Japan. July, 2014.

Taniguchi M. NKT cell-mediated adjuvant cell therapy on lung cancer and head and neck cancer. Federation of Clinical Immunology Societies 2014, Chicago, USA. June, 2014.

Taniguchi M. NKT cells as an ideal anti-tumor immunotherapeutic. The 54th Annual Meeting of the Japanese Respiratory Society, Osaka, Japan. April, 2014.

Laboratory for Immunotherapy

Team Leader: Shin-ichiro Fujii

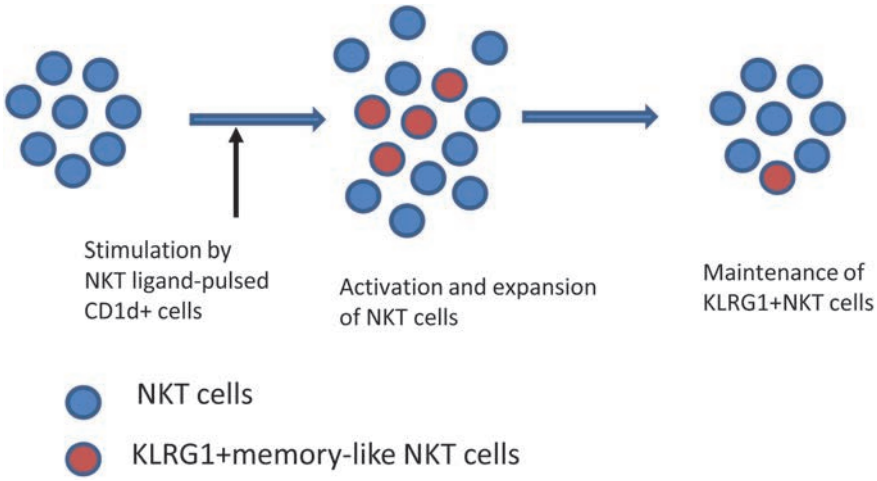


Figure: Generation of KLRG1⁺ memory-like NKT cells
When NKT cells are stimulated by NKT ligand-pulsed CD1d⁺ cells, the number of NKT cells transiently increases.
After returning to the original steady state, some KLRG1⁺ memory-like NKT cells survived.

The aims of the laboratory are to extend our basic studies for advancing immunotherapy and translational research (TR), from basic studies back and forth to the bedside in the field of cancer. To accomplish this, we have been conducting three NKT cell-related TR projects using the synthetic glycolipid, α-galactosylceramide (α-GalCer). When NKT cells are activated in this manner, they acquire unique immunostimulatory features that include the rapid production of IFN-γ and NK cell activation, followed by innate immunity-mediated antitumor effects. Although NKT cells belong to the innate immune system, we recently discovered a new type of NKT cell, the memory like KLRG1⁺NKT cell, which can survive for a long period after activation (Fig.).

As a first TR project, we have attempted to establish a strategy linking innate and adaptive immunity. For this purpose, we have been studying the process of activation of adaptive immunity through full maturation of DCs that occurs soon after the activation of NKT cells *in vivo*. In addition, we are investigating novel delivery systems that have the potential to enhance antitumor immunity. We have developed artificial adjuvant vector cells as a new type of drug delivery system composed of NKT ligand and tumor associated antigen, linking innate and adaptive immunity. We are also confirming the induction of memory NKT cells using this new type of vaccine. Under the RIKEN translational program and Tokyo University translational research program, we are making efforts to work toward preclinical studies that will ultimately lead to clinical trials. Second, we have been working in a collaborative study with the RIKEN iPS-group to establish iPS-NKT cells. In this project, we focus on the preparation of primary NKT cells as the starting material for generating NKT cell-derived iPS cells and on the analysis of the function of iPS-derived NKT cells. Third, we have been collaborating a joint clinical phase I / IIa study of NKT cell therapy with the National Hospital Organization (NHO) for early stage of lung cancer patients after surgical tumor resection. In this study, we play an important role in the analyses of immune responses.

Recent Major Publications

Shimizu K, Sato Y, Shinga J, Watanabe T, Endo T, Asakura M, Yamasaki S, Kawahara K, Kinjo Y, Kitamura H, Watarai H, Ishii Y, Tsuji M, Taniguchi M, Ohara O, Fujii S. KLRG⁺ invariant natural killer T cells are long-lived effectors. ***Proc Natl Acad Sci U S A*** 111, 12474–9 (2014)

Shimizu K, Mizuno T, Shinga J, Asakura M, Kakimi K, Ishii Y, Masuda K, Maeda T, Sugahara H, Sato Y, Matsushita H, Nishida K, Hanada K, Dorrie J, Schaft N, Bickham K, Koike H, Ando T, Nagai R, Fujii S. Vaccination with antigen-transfected, NKT cell ligand-loaded, human cells elicits robust in situ immune responses by dendritic cells. ***Cancer Res*** 73, 62–73 (2013)

Shimizu K, Asakura M, Shinga J, Sato Y, Kitahara S, Hoshino K, Kaisho T, Schoenberger SP, Ezaki T, Fujii S. Invariant NKT cells induce plasmacytoid DC cross-talk with conventional DCs for efficient memory CD8+ T cell induction. ***J Immunol*** 190, 5609–19 (2013)

Invited Presentations

Fujii S. Development of a New Type of Cancer Vaccine "artificial Adjuvant Vector Cells (aAVC)". Seminar in Dep. Cartilage & Bone Regeneration (Fuji-Soft), Graduate School of Medicine, The University of Tokyo, Tokyo, Japan. December, 2014.

Fujii S. Immunological memory and antitumor immunity elicited by artificial adjuvant vector cells for cancer immunotherapy. The 43rd Annual Meeting of the Japanese Society for Immunology (JSI), Kyoto, Japan. December, 2014.

Fujii S. "Development of cancer vaccine triggered by innate immunity". Seminar in Chiba University Graduate School of Medical and Pharmaceutical Sciences, Chiba, Japan. August 2014.

Fujii S. Development of a New Type of Cancer Vaccine Linking Innate and Adaptive immunity. Seminar in Jichi Medical University Graduate School of Medicine, Tochigi, Japan. July, 2014.

Fujii S. Development of a Novel Type of Cancer Vaccine Linking Innate and Adaptive immunity. The 22nd International Symposium on Molecular Cell Biology of Macrophages (MMCB2014), Kobe, Japan. June, 2014.

Laboratory for Vaccine Design

Team Leader: Yasuyuki Ishii

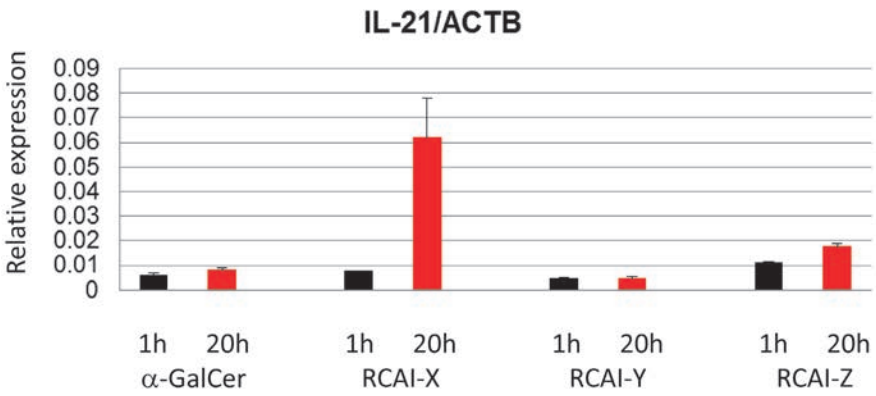


Figure: IL-21 expression by NKT cells treated with liposomal α-GalCer analogues
C57BL/6 mice were injected intravenously with liposomal α-GalCer analogues, RCAI-X, Y or Z. Splenic NKT cells were sorted 2 or 20 h after the injection. IL-21 mRNA was assessed using a quantitative PCR method.

Recent Major Publications

Shimizu K, Sato Y, Shinga J, Watanabe T, Endo T, Asakura M, Yamasaki S, Kawahara K, Kinjo Y, Kitamura H, Watarai H, Ishii Y, Tsuji M, Taniguchi M, Ohara O, Fujii S. KLRG^{+} invariant natural killer T cells are long-lived effectors. ***Proc Natl Acad Sci U S A*** 111,12474–9 (2014)

Sakurai T, Inamine A, Iinuma T, Funakoshi U, Yonekura S, Sakurai D, Hanazawa T, Nakayama T, Ishii Y, Okamoto Y. Activation of invariant natural killer T cells in regional lymph nodes as new antigen-specific immunotherapy via induction of IL-21 and IFN-γ. ***Clin Exp Immunol*** 178, 65-74 (2014)

Hirai T, Ishii Y, Ikemiyagi M, Fukuda E, Omoto K, Namiki M, Taniguchi M, Tanabe K. A Novel approach inducing transplant tolerance by activated invariant natural killer T cells with costimulatory blockade. ***Am J Transplant*** 14, 554–67 (2014)

Natural killer T (NKT) cells perform immunoregulatory roles, such as the suppression of IgE responses, as well as having adjuvant roles in host defense. Although the mechanism of immune activation by NKT cells is well understood, that of immune suppression remained unclear. In our previous studies, we showed that a liposome formulation of α-galactosylceramide (α-GalCer), a representative NKT cell ligand, could be preferentially delivered to a splenic B220-positive cell subset. As this subset included IgE-expressing B cells, IL-21 derived from NKT cells played a role in the induction of B cell-specific apoptosis, and then IgE isotype-specific suppression. To develop this activity into an IgE-suppressive drug, we screened α-GalCer analogue compounds by assessing IL-21 expression by NKT cells derived from mice injected intravenously with the liposomal compounds. Three compounds, RCAI-X, Y and Z were selected. The general toxicology and genetic toxicology were analyzed for each liposome formulation. All liposomal compounds were well tolerated in the mice when given up to three times in one week at intravenous injection doses up to 5 mg/kg over two weeks (6 total doses). In these pharmacological studies, liposomal RCAI-X enhanced IL-21 expression by NKT cells better than the other compounds and showed better suppression of *in vivo* secondary IgE antibody responses. Based on these results, we have decided to develop RCAI-X as the first candidate IgE-suppressive drug.

Laboratory for Allergic Disease

Team Leader: Toshiaki Kawakami

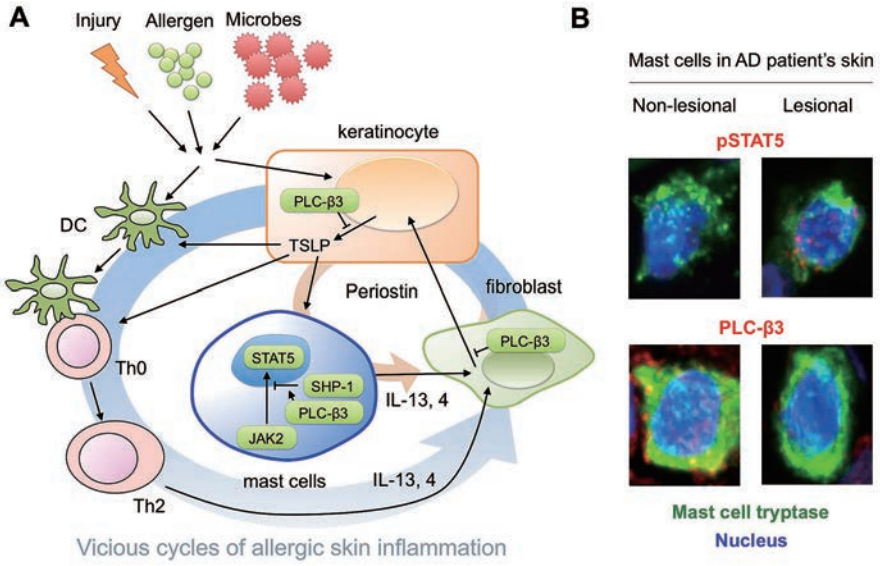


Figure: Hypothetical vicious cycle of allergic skin inflammation
(A) Allergen-specific T_H2 cells stimulate production/secretion of periostin by fibroblasts. Periostin in turn then stimulates keratinocytes to produce and secrete TSLP and other inflammatory cytokines, forming a feed-forward loop to exacerbate the skin allergic inflammation. Once sustained overexpression of TSLP is established, mast cells may play a more important role in persistent dermatitis than T_H2 cells. PLC-β3 can regulate activities of the cellular elements of this network, such as proliferation of mast cells, periostin production/secretion by fibroblasts and TSLP production/secretion by keratinocytes. (B) An inverse correlation between phosphorylated STAT5 and PLC-β3 expression levels was observed in mast cells of a human atopic dermatitis patient. DC, dendritic cell; T_H0, naïve $CD4^+$ T cells.

Recent Major Publications

Kawakami T, Kashiwakura J, Kawakami Y. Histamine-releasing factor and immunoglobulins in asthma and allergy. ***Allergy Asthma Immunol Res*** 6, 6–12 (2014)

Ando T, Xiao W, Gao P, Namiranian S, Matsumoto K, Tomimori Y, Hong H, Yamashita H, Kimura M, Kashiwakura J, Hata TR, Izuhara K, Gurish MF, Roers A, Rafaels NM, Barnes KC, Jamora C, Kawakami Y, Kawakami T. Critical role for mast-cell Stat5 activity in skin inflammation. ***Cell Rep*** 6, 366–76 (2014)

Ando T, Matsumoto K, Namiranian S, Yamashita H, Glatthorn H, Kimura M, Dolan BR, Lee JJ, Galli SJ, Kawakami Y, Jamora C, Kawakami T. Mast cells are required for full expression of allergen/SEB-induced skin inflammation. ***J Invest Dermatol*** 133, 2695–705 (2013)

Invited Presentations

Kawakami T. Critical role of mast-cell Stat5 in atopic dermatitis, Lessons from studies on phospholipase C-beta3. Academy of Immunology and Microbiology (AIM) Workshop on Immunology 2014, Pohang, Republic of Korea. November, 2014.

Kawakami T. Roles of Stat5 in atopic dermatitis and hematopoietic malignancy. The 14th Annual Meeting of The Korean Atopic Dermatitis Association, Seoul, Republic of Korea. November, 2014.

Kawakami T. Introduction to mast cell biology. IMMUNOLOGY 2014, Block Symposium: Mast cell biology, Pittsburgh, USA. May, 2014.

Kawakami T. Two tales of allergic inflammation. Microbiology/Immunology Seminar Series, Montana State University, Bozeman, USA. April, 2014.

Kawakami T. the Breakfast Seminar entitled "New insights into IgE biology". The Annual Meeting of The American Academy of Allergy, Asthma and Immunology, San Diego, USA. March, 2014.

Role of histamine-releasing factor (HRF) in allergic diseases

HRF is a cytokine-like protein that can stimulate histamine release and cytokine (IL-4 and IL-13) production/secretion from IgE-sensitized basophils and mast cells. HRF activities are found in bodily fluids during the late phase of allergic reactions. We demonstrated that some, but not all, IgE and IgG molecules interact with HRF with low affinity. By mapping the binding sites on both HRF and IgE/IgG molecules, we developed competitive inhibitors of HRF-IgE (or IgG) interactions. Using these inhibitors, we showed that HRF promotes allergic inflammation in mouse models of anaphylaxis and asthma (Kashiwakura et al., 2012). For the past two years, we have been studying the role of HRF in food allergy. Using an ovalbumin-induced food allergy model, which is mast cell-, IgE-, and FcεRI-dependent, our numerous experiments have shown that HRF is involved in the promotion of allergic reactions including diarrhea (unpublished). Our more recent experiments treating diarrheic mice with several HRF inhibitors reduced both the occurrence and clinical severity of diarrhea induced upon allergen challenge. These results strongly support the efficacy of the HRF inhibitors as potential therapeutics for food allergy. We have also observed reduced levels of HRF-reactive IgGs in sera of food allergy patients after successful oral induction of tolerance. We also isolated four HRF-related human mRNA sequences, all of which could interfere with HRF-Ig interactions (unpublished).

Pathogenic mechanisms of atopic dermatitis (AD)

AD is a chronic pruritic inflammatory skin disease. In the AD project, we have been studying its cellular and molecular mechanisms using our previously established *in vivo* AD induction model and the spontaneously occurring AD-like skin lesions in phospholipase C (PLC)-β3-deficient mice. As we recently showed the importance of PLC-β3-mediated Stat5 regulation in mast cells in causing AD (Ando et al., 2014; Fig.), we are now studying whether Stat5 inhibitors can be utilized as new therapeutics for atopic dermatitis.

RIKEN-TORII Joint Research Team

Team Leader: **Masaru Taniguchi**

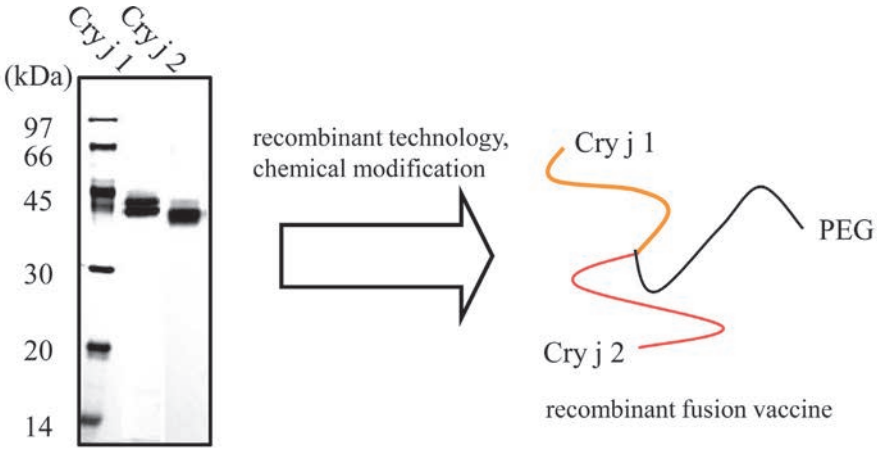


Figure: Structure of the recombinant fusion vaccine for Japanese cedar pollinosis
Recombinant technology is used to conjugate two major allergens from Japanese cedar pollen, Cry j 1 and Cry j 2, and it is further chemically modified with polyethylene glycol to improve solubility.

Invited Presentations

Fujimura T. Development of immunotherapeutic vaccine for Japanese cedar pollinosis and findings of therapeutic biomarkers for allergnen-specific immunotherapy. The 3rd Workshop of Hiroshima Research Center for Healthy Ageing (HiHA), Hiroshima, Japan. December, 2014.

Fujimura T. Allergen-specific immunotherapy for pollinosis, up-to-date. The 7th Basic Seminar of Society of Atopy, Allergy, and Immunology in Veterinary Medicine, Tokyo, Japan. August, 2014.

Antigen-specific immunotherapy is considered to be the only curative treatment for allergy. In 2014, a standardized Japanese cedar pollen extract for sublingual immunotherapy was commercially provided by TORII Pharmaceutical Co., Ltd. as a curative vaccine for Japanese cedar pollinosis. However, only a crude extract from Japanese cedar pollen has been approved for clinical use by the Ministry of Health, Labour and Welfare in Japan. Vaccines using allergoids and modified Cry j 1, a major allergen of Japanese cedar pollen, have been developed and used for pre-clinical trials. However, none of them has been commercially available for medical use due to poor clinical outcomes at later stage clinical trials or to the failure to find a cooperative pharmaceutical company to introduce them into the market. To fill the critical gap between basic research and later stage drug development, RIKEN and TORII Pharmaceutical Co., Ltd. set up a joint research laboratory in IMS-RCAI in May 2010 and started to develop a vaccine.

For this vaccine, recombinant technology is used to conjugate two major allergens from Japanese cedar pollen, namely Cry j 1 and Cry j 2, and then it is further modified with polyethylene glycol to improve solubility. Systemic injections of the vaccine into Cry j 1 or Cry j 2-sensitized mice prevented the increase of serum antigen-specific IgE normally observed following systematic or local sensitization with native Cry j 1 or Cry j 2 in dose-dependent manner. Furthermore, the vaccine prevented the increase of Cry j 1 and Cry j 2 specific IgE after local sensitization with Japanese cedar pollen in monkeys. We thus confirmed animal POC (proof of concept) for this vaccine.

Drug Discovery Antibody Platform Unit

Unit Leader: Toshitada Takemori

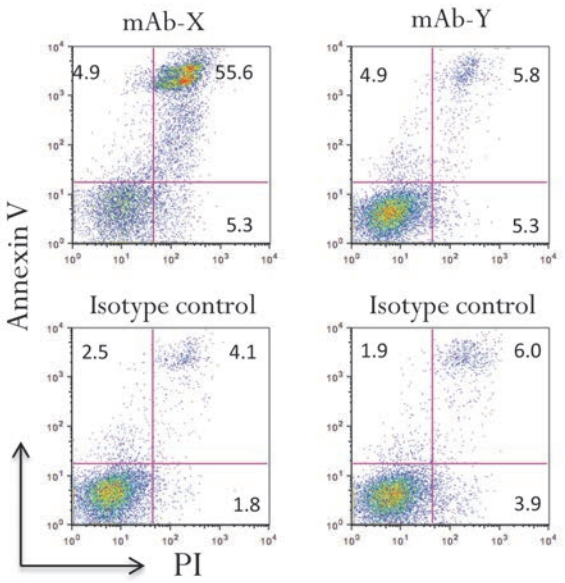


Figure: A novel screening system for monoclonal antibodies (mAbs)
mAb-X has the activity to kill the target cell independent of ADCC and CDC, as defined by FACS analysis after 4 h incubation *in vitro*. mAb-Y does not have such activity.

Our laboratory is linked to the RIKEN Program for Drug Discovery and Medical Technology Platforms (DMP). The aim of DMP is to contribute to the identification of new treatments for cancer and other diseases by promoting collaboration within RIKEN for the development of innovative new pharmaceuticals and medical technologies.

The use of monoclonal antibodies (mAbs) for cancer therapy has achieved significant success in recent years. Tumor cell killing is mediated through direct action of the antibody (through receptor blockade or agonist activity), complement-dependent cytotoxicity (CDC) and/or antibody-dependent cellular cytotoxicity (ADCC). The antibody Fc is essential for mediating tumor cell killing through CDC and ADCC, but the antibody V-region is also important for the killing activity with respect to the recognition sites and affinity for the target molecules.

In this context, we have establish a system that enables us to easily and simply evaluate the ADCC and CDC activities of mAbs in a large number of culture supernatants at any time point during hybridoma selection in HAT medium. Accordingly we are able to preferentially select the best functional clones during the initial hybridoma culture, prior to mAb purification. Furthermore, we established a mouse model to evaluate the anti-cancer activity of mAbs selected by this *in vitro* system.

Our laboratory is now developing mAb drugs for glioma and acute myeloid leukemia, AML. We are using several model systems to clarify the activity of mAbs obtained in our laboratory in terms of whether they could be applicable in medical therapies, prior to further analysis on the clinical side. In addition, we are analyzing the mechanism of lymphoma-specific killing by a particular mAb without ADCC and CDC activities, which is independent of apoptosis and necrosis pathways. This task, if successful, may provide a new route for cancer cell eradication.

Recent Major Publications

Takemori T. Generation of memory B cells. In ***Molecular Biology of B Cells***, 2nd Ed. Alt FW, Honjo T, Radbruch A and Reth M (eds.), Academic Press, London, UK, 227–32 (2014)

Takemori T, Kaji T, Takahashi Y, Shimoda M, Rajewsky K. Generation of memory
B cells inside and outside germinal centers. ***Eur J Immunol*** 44, 1258–64 (2014)

Kaji T, Furukawa K, Ishige A, Toyokura I, Nomura M, Okada M, Takahashi Y, Shimoda M, Takemori T. Both mutated and unmutated memory B cells accumulate mutations in the course of the secondary response and develop a new antibody repertoire optimally adapted to the secondary stimulus. ***Int Immunol*** 25, 683–95 (2013)

Invited Presentations

Takemori, T. CD4 memory T cells develop and acquire functional competence by sequential cognate interactions and stepwise gene regulations. Symposium on the International Leibniz Research Cluster ImmunoMemory: Organization of Immunological Memory, Berlin, Germany. November, 2014.

Central Facilities

Central Facilities in IMS provide all researchers in the Center with access to the most advanced equipment and technologies. Central Facilities consist of four sections; the FACS Laboratory managed by Dr. Takashi Saito, the Confocal Laboratory managed by Dr. Takaharu Okada, the Genomics Laboratory managed by Dr. Osamu Ohara, and the Animal Facility managed by Dr. Haruhiko Koseki.

FACS Laboratory

The FACS Lab provides a range of support for flow cytometry and cell sorting, techniques that are essential for nearly all immunological experiments. The FACS Lab has added a new FACS Aria and is no longer using the older FACS Vantages. In addition to FACS, the lab installed ImageStreamX, a device that combines flow cytometry with the visual detail of microscopy in a single platform, and upgraded CyTOF2, a mass-spectrometry-based cytometer that has the potential for analyzing more than 30 markers simultaneously with metal-labeled antibodies.

For the users of the FACS machines (cell analyzers and cell sorters), Tomomi Aoyama and Noriko Yoza offer various services: (1) Technical support and training: In 2014, the facility offered 16 technical courses (6 for cell sorting and 10 for cell analysis). Courses were held at 3 different levels, Calibur basic, Canto II and Aria basic. A total of 58 researchers took the courses in 2014. (2) Cell sorting operation service: The FACS Lab provides a cell sorting operation service, in which researchers can ask an experienced operator to conduct the sorting experiment. In 2014, the lab provided 266 such operation services. (3) Management/ maintenance of FACS machines: FACS machines are available for registered users 24 hours a day and reservations are accepted up to one month in advance through an internal website. In addition to the in-house FACS Lab staff, engineers from Becton Dickinson visit once a week to provide maintenance and technical support.

Table: Instruments in the FACS Lab.

Machine types	Machines	# of machines
FACS cell analyzer	Calibur Canto II	4 2
FACS cell sorter	Aria II Aria III	3 2
Mass-cytometer	CyTOF2	1
Imaging flow cytometer	ImageStreamX	1

Confocal Laboratory

The Confocal Lab. provides equipment for cell and tissue imaging, and coordinates technical support. There are seven laser-scanning fluorescence microscopes and a super-resolution microscope available to researchers at IMS.

1. Inverted Leica SP2 system with visible lasers for single-photon excitation and a femtosecond Ti:Sa laser for two-photon excitation.
2. Inverted Leica SP2 system with visible lasers for single-photon excitation including a 405 violet laser. This microscope is equipped with a chamber system that controls CO_2 concentration, temperature and humidity for live cell imaging.
3. Inverted Leica SP5 system with visible lasers for single-photon excitation including a 405 violet laser.
4. Upright Leica SP2 system with visible and UV lasers for single-photon excitation.
5. Inverted Leica SP8 system with visible lasers for single-photon excitation. SP8 is Leica's newest system with improved optics.
6. Upright Leica SP5 system with two femtosecond Ti:Sa lasers for two-photon excitation. This system utilizes resonant scanners that enable high-speed acquisition of large z-stacks for live tissue imaging.
7. Inverted Leica SP8 system with two femtosecond Ti:Sa lasers for two-photon excitation. This system is equipped with two types of scanners (resonant and galvano) and hybrid detectors with high sensitivity and low background noise. One of the two Ti:-Sa lasers is connected to an optical parametric oscillator (OPO) that enables two-photon imaging by long wavelength excitation.
8. Inverted Nikon N-SIM/N-STORM super-resolution microscope for dual color imaging.

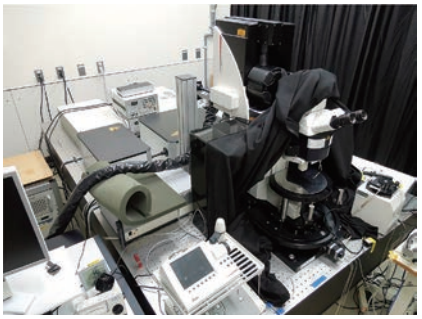


Photo: Upright SP5 two-photon microscope (#6)

Genomics Laboratory

The Laboratory for Integrative Genomics also serves as a technical support service lab that provides genome- and proteome-wide analysis for scientific research groups in the Center for Integrative Medical Sciences (IMS). They offer a variety of services to suit the needs of different labs. These include DNA sequencing, proteomics analysis, multiplex suspension array, DNA microarray (Affymetrtix), cDNA/Genomic clone distribution, and Primer/labeled probe distribution for qRT-PCR analysis of immune cells (Table). Supplying advanced technologies on demand, they provide comprehensive interrogation of the nucleic-acid based information in a cell at single-base resolution with the Illumina HiSeq1500 and as well as proteomic approaches using the AB SCIEX TripleTOF 5600. Using the unbiased sequencer approach, the Genomics Laboratory has interrogated for: transcription units, mapping/genome annotation, alternative splice sites, and transcription factor binding sites. Our mass spectrometry system will make it possible to use quantitative proteomic approaches in various immunological studies. These technologies will help to reveal additional hidden features of the dynamic genomic and proteomic landscape that are regulated by both genetic and epigenetic pathways in all organisms.

Table: Services provided by the Genomics Lab in 2014

Next-generation DNA sequencing	**# of samples**	**# of teams**
RNA-sequencing	739	9
Chip-sequencing	158	6
Others (Exome etc)	20	2
Total	917	12
Proteimics	**# of samples**	**# of teams**
Mass Spectrometry Analysis	1	1
Multiplex suspension array	1,968	13
Affymetrix Genechip (Exon array, Gene array, miRNA array)	**# of samples**	**# of teams**
Human	47	1
Mouse	38	3
Total	85	4
Sanger DNA sequencing	**# of samples**	**# of teams**
36cm capillary	6,640	17
50cm capillary	5,536	17
Total	12,176	21
cDNA clone delivery	**# of clones**	**# of teams**
	23	3
Primer/labeled probe delivery	**# of sets**	**# of teams**
	105	1

Animal Facility

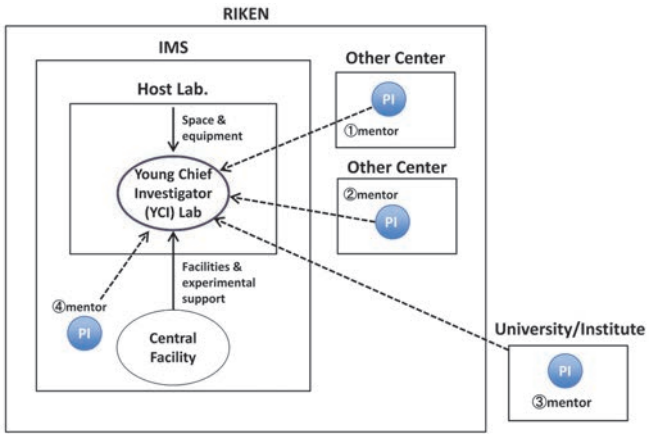
The animal Facility maintains over 50,000 mice in the SPF area, which also contains 550 germ-free or gnotobiotic mice in the Vinyl Isolator rooms and Vinyl Isolator bio-bubble rooms for "humanized mice", and 1,500 mice in an isolated area. Mouse lines are introduced into the SPF area by a combination of *in vitro* fertilization (IVF) and embryo transfer and 704 lines of cryostocks of genetic resources have been generated. The animal facility also maintains relatively large colonies of several commonly used strains such as NOD/SCID/Il2rγKO mice, Rag1KO and cre deleters, and provided them to users on demand. The facility has also provided technical assistance to generate knockouts (87 lines) and transgenic mice (10 lines). The animal facility has attempted sanitization of knockout mice and built a system that creates germ-free mice (12 lines).

In addition, the facility has launched a new activity to improve the efficacy of transplantation of human hematopoietic stem cells into NOD/SCID/Il2rγKO mice by "humanizing" the host strain. For this purpose, they have introduced large genomic fragments containing human genes encoding MHC, cytokines, adhesion molecules, virus receptors and others into NOD/SCID/Il2rγKO mice. Up to now, they have established 15 BAC transgenic mice and 4 knock-in mice with confirmed expression of human genes on a C57BL/6 background and begun back-crossing these mice onto the NOD/SCID/Il2rγKO mice using the speed-congenic method.

Beginning in April of this year, the animal facility has begun to generate KO and KI mice using the CRISPR/Cas system (68 constructs prepared). In addition, they have been breeding germ-free C57BL/6N mice and started providing those to users beginning in June (20 - 40mice/month). Lastly, they have also generated an internally available database for genetic resources.

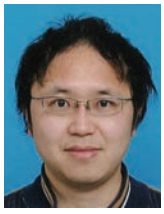
Photo: Creation of germ-free mouse

Young Chief Investigator Program



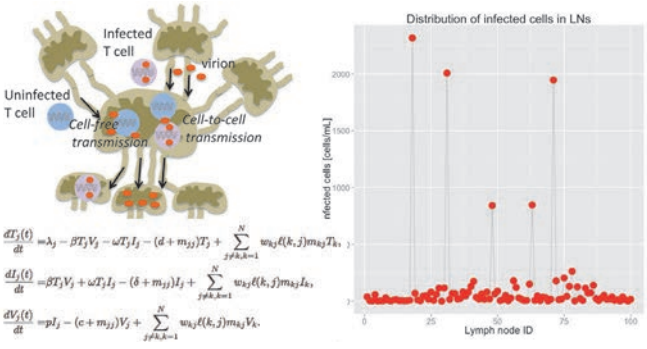
The Young Chief Investigator Program aims to provide a career path for young investigators who conduct multidisciplinary research that will bridge immunology with other research fields. In this program, the selected Young Chief Investigator (age below 40) will head an independent research laboratory but will have an access to mentoring by multiple senior specialists in related research fields. Mentors provide guidance for experimental design, preparation of papers and presentations, promotion of international visibility, and obtaining research funding. The YCI laboratory will also share space, equipment and facilities with a host laboratory in IMS (Fig.) The YCI Program Committee considers necessary changes in the Center's support for each YCI and discusses the relevance and value of each YCI research project as part of the core research projects at IMS.

There will be an initial 5-year appointment with the possibility of extending for an additional 2 years after evaluation by the Director and an internal committee. At that point, a Young Chief Investigator can leave IMS to take a position at another institution or be promoted to another type of position within IMS.



YCI Laboratory for
Mathematical Modeling of Immune System

Young Chief Investigator: **Shinji Nakaoka**



We are undertaking several mathematical works that are indispensable for construction of multi-scale mathematical models. In this year, we especially focused on HIV infection in a lymph node (LN) network. We proposed a mathematical model which describes infection of CD4 positive T cells within a lymph node. Migration of T cells via blood and the lymphatic system is incorporated to simulate the spread of infection *in vivo*. Upon the *in silico* construction of a lymph node network, a volume proportionality assumption is employed to distinguish kinetic heterogeneity of the infectious process. The basic reproduction number defined for the next generation matrix associated with our main model is calculated to quantitatively characterize the spread of HIV infection. We then simulate combinational multi-drug therapy to investigate the effects of reverse-transcriptase and protease inhibitors on HIV infection. One important and interesting finding from our theoretical study is the existence of the limit of drug treatment that was discovered by our incorporation of the network structure of lymph nodes. This limit might partly explain why HIV can persist despite the successive administration of combinational multi-drug treatment.

Figure: HIV infection in a lymph node network
A schematic representation of HIV dynamics in a LN network (left) and the expected distribution of infected cells (right)

YCI Laboratory for
Stem Cell Competency
Young Chief Investigator: Hayato Kaneda

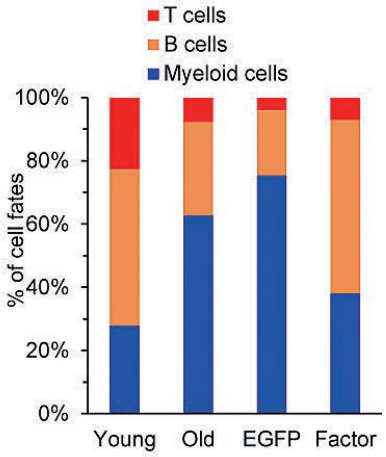


Figure: Correction of the reduced B cell ratio in the peripheral blood of old mice by lentiviral transduction of a young MSC-secreting factor
Mesenchymal stem/stromal cells (MSCs) are well known to secrete a variety of homeostatic factors. However, no critical factors had been identified yet. We identified one of the young MSC-secreting factors and investigated its effects on age-related dysfunctions by lentiviral transduction into old mice. We found that the factor restored the B cell ratio to the young blood level (Factor) while the control vector (EGFP) had no effect.

Adult tissue stem cells (TSCs) become impaired in their functions with age. TSC dysfunction and decreased regenerative capacity are involved, at least in part, in disturbances of tissue homeostasis, e.g., inefficient muscle repair, reduced bone mass, neurodegenerative diseases, and dysregulation of hematopoiesis. Therefore, the restoration of TSC functions is expected to contribute to recovery of tissue homeostasis and improvements in our health.

Previously, we identified the "competence change", which is responsible for the responsiveness of neural stem/progenitor cells (NSPCs) to extrinsic signals (Naka et al., Nat Neurosci, 2008). Moreover, further investigation revealed that competence regulation enabled us to control the neurogenic-to-gliogenic transition and restore neurogenic potential in developmentally-progressed gliogenic NSPCs (Naka-Kaneda et al., Proc Natl Acad Sci USA, 2014). Based on these findings, we have been investigating stem cell aging. Competence regulation is also involved in the aging of other TSCs, such as the decline in lymphopoiesis by hematopoietic stem cells (Fig.) and in the osteogenesis ability of mesenchymal stem/stromal cells. We aim to elucidate the central molecular machinery of stem cell aging and its influence on tissue homeostasis and, in turn, to develop a method for functional recovery of aged stem cells and restoration/maintenance of tissue homeostasis.

YCI Laboratory for
Immune Regeneration
Young Chief Investigator: Tomokatsu Ikawa

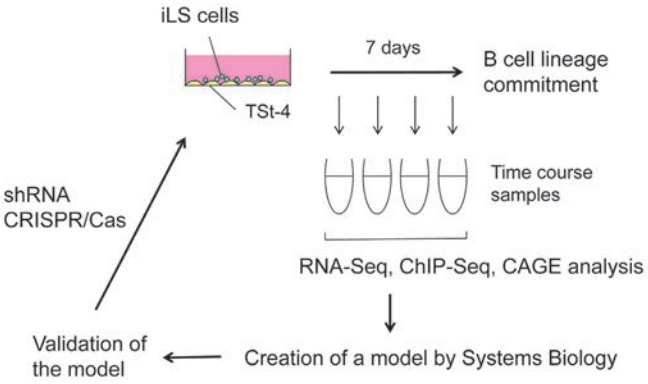


Figure: Analytical cycle to identify the core transcriptional network controlling B cell fate specification
We have established multipotent progenitors that have self-renewal activity. This was done by overexpressing Id3 in hematopoietic stem/progenitor cells and culturing the cells under B cell differentiating conditions. Id3 suppresses E2A activity and the multipotent progenitors acquire self-renewal capacity. The cells extensively proliferate for at least several months, still maintaining their multipotency. We named the cells, induced Leukocyte Stem (iLS) cells. Using iLS cells we are examining the transcriptional network during B lineage commitment.

T, B and NK lymphocytes are generated from pluripotent hematopoietic stem cells (HSCs) through a successive series of lineage restriction processes. Transcription factors (TFs) play a key role in regulating lineage-associated gene programs. Although many essential TFs, such as PU.1, Ikaros, GATA3, TCF-1, Bcl11b, E2A, EBF1 and Pax5 have been implicated in regulating the cell fate choice of lymphoid lineages, molecular mechanisms underlying the generation of these patterns during cell fate determination remain unexplored because of the lack of suitable experimental systems.

We have recently established stable multipotent progenitor cells, termed induced Leukocyte Stem (iLS) cells, which can be used to examine gene regulatory networks during lymphoid lineage specification from HSCs (Fig.). We have examined the transcriptional network during B cell commitment. B lineage commitment was completed within 7 days using the iLS system. Time-course gene expression analysis revealed the key TFs, EBF1, Pax5 and FoxO1 were upregulated 48 hrs after the induction. Rapid upregulation/downregulation of TFs was found before the induction of the key factors. The early responding TFs exhibited wave-like expression patterns, indicating that a successive transcriptional cascade is essential for B cell fate specification. This inducible culture system is useful for elucidating the mechanisms that orchestrate cell fate specification, commitment and differentiation during lymphocyte development. It can also be applied for *ex vivo* expansion of human hematopoietic stem/progenitors, which will be required for immune cell therapy or transplantation of HSCs.

YCI Laboratory for
Quantitative Omics
Young Chief Investigator: Katsuyuki Shiroguchi

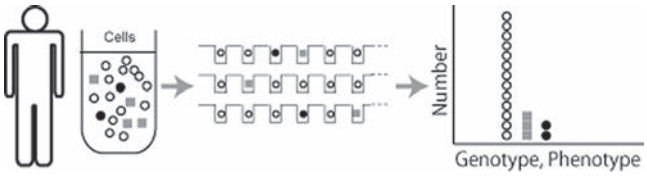
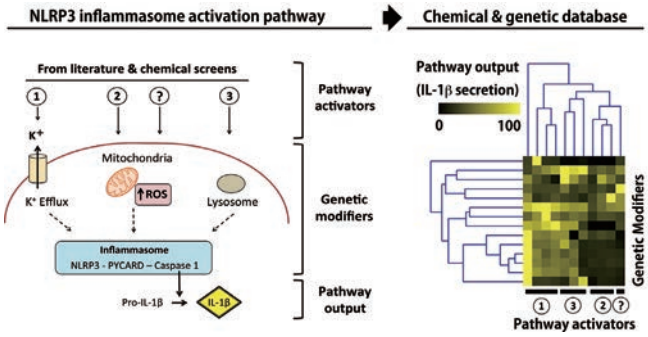


Figure: Identification of the overall state of an individual by system-wide measurements with single cell resolution

Quantitative Omics at the Single Cell & Single Molecule Level for Biological and Medical Sciences

A single cell may affect the behavior of a cell population, and the state of tissues and individuals. For example, at the onset of a disease, homeostasis is not destroyed simultaneously in an entire population of cells, but instead begins at the single cell level. In order to understand such phenomena at the molecular level, we wish to visualize the distribution of cell states by accurate system-wide measurements with single molecule and/or single cell resolution. To visualize the distribution and its shift depending on the cell states, we are working on the development of highly accurate quantification methods.

We have been developing a molecular barcoding method in order to perform digital counting of RNA molecules genome-wide using a next generation sequencer. We are also developing a cell barcoding method to measure many cells at a time with single cell resolution. These techniques allow us to perform high throughput and high resolution analyses, which enable us to identify the state of biological systems based on the cell states and the cell number distribution of those cells. We are also working on applications to contribute to medical sciences.



YCI Laboratory for
Cellular Bioenergetic Network
Young Chief Investigator: Toshimori Kitami

Figure: Chemical and genetic dissection of the NLRP3 inflammasome pathway
We hope to identify genes and chemical compounds involved in NLRP3 inflammasome activation as a research tool for dissecting the role of mitochondria in this complex disease pathway.

The overarching goal of our laboratory is to understand the role of cellular metabolism in the pathogenesis of complex diseases. Research over the past decades has shown that monogenic mutations in metabolic pathways cause a wide variety of human diseases. However, more recent studies have highlighted the role of cellular metabolism in the development of a wide variety of complex human diseases. Our laboratory in particular has been studying the function of mitochondrial energy metabolism, which is associated with neurodegeneration, cardiovascular disease, type 2 diabetes, and aging. We hope to identify novel pathways that restore or improve mitochondrial function through genetic and chemical screens and to examine their potential therapeutic value using genetically engineered mouse models and unique chemical probes.

Towards our goal, we have begun to explore the role of mitochondria in the innate immune pathway called the NLRP3 inflammasome, which is involved in a variety of complex diseases. The NLRP3 inflammasome is activated by changes in cell physiology, including mitochondrial damage, although the molecular players involved have not been fully elucidated. We hope to leverage our expertise in high-throughput screens to systematically identify genes and pathways involved in NLRP3 inflammasome activation and to accurately place mitochondria in the context of this important disease pathway.

Award winners 2014


Photo 1


Photo 2


Photo 3


Photo 4


Photo 5


Photo 6


Photo 7


Photo 8


Photo 9


Photo 10


Photo 11


Photo 12

On April 15th, 2014, the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) awarded a Commendation for Science and Technology to **Shigeo Koyasu** (Photo 1), Director of IMS and Group Director of the Laboratory for Cell Systems. He won the Research Category Prize for his studies on innate immunity against parasite infection. This prize is awarded to investigators whose research is highly original and contributes to the science and technology of Japan.

The Japan Society of Human Genetics (JSHG) awarded the Japan Society of Human Genetics (JSHG) Award to **Michiaki Kubo** (Photo 2), Deputy Director of IMS and Group Director of the Laboratory for Genotyping Development. He received the award for developing the foundation of genomic medicine using Genome-wide association study (GWAS) and its application to practice.

Kazuhiko Yamamoto (Photo 3), Team Leader of the Laboratory for Autoimmune Diseases, received the 12th Takamine Memorial Daiichi Sankyo Prize on June 24th, 2014. He received the prize for his studies of antigen-specific immune responses in human autoimmune diseases. This prize is given to an active researcher who is achieving remarkable feats in the progress and development of the life sciences, particularly in basic research fields relating to the prevention and cure of disease as well as research with clear clinical applications.

Yamamoto received the Medical Award of The Japan Medical Association on November 1st, 2014, for his research of the molecular analyses of autoimmune diseases. He also received the 1st Human Immunology Research Award from the Japanese Society for Immunology for his study of human autoimmune diseases. This award is given to an outstanding researcher who has contributed to the human immunology field.

Yuta Kochi (Photo 4), Senior Research Scientist in the Laboratory for Autoimmune Diseases, received the MEXT Prize for Young Investigators. This prize is awarded to young scientists (under 40 years of age) in recognition of their creative and original research and outstanding ability to develop scientific research projects. He received the award for his research on genetic factors among Japanese that affect their susceptibility to rheumatoid arthritis.

Hiroaki Kitano (Photo 5), Group Director of the Laboratory for Disease Systems Modeling was selected as an ISSB (International Society for Systems Biology) Fellow for his outstanding and pioneering contributions to establish systems biology

Tomomitsu Hirota (Photo 6), Research Scientist in the Laboratory for Respiratory and Allergic Disease received The Award for Young Scientist from The Japan Society of Human Genetics for his study, "Identification of susceptibility loci for allergic diseases by GWAS".

Kenya Honda (Team Leader, Photo 7), **Koji Atarashi** (Visiting Researcher, Photo 8), and **Takeshi Tanoue** (Special Postdoctoral Researcher, Photo 9) of the Laboratory for Gut Homeostasis, received the second prize in the Gottfried Wagener Prize 2014 competition for their isolation of intestinal bacterial strains that strongly affect the host immune system.

Honda also received the Tatsuji Nomura Prize 2014 from The Keio Medical Society on November 7th, 2014, for the identification and isolation of potent immune modulatory bacteria from the gut microbiota.

Masayuki Amagai (Photo 10), Team Leader of the Laboratory for Skin Homeostasis, received the Ogawa-Seiji Memorial Award from the Lydia O'Leary Memorial Pias Dermatological Foundation on May 31, 2014.

Shiro Ikegawa (Photo 11), Team Leader of the Laboratory for Bone and Joint Diseases, received the Russell A. Hibbs Best Basic Research Award from the Scoliosis Research Society for his presentation "A new genetic locus increases risk of idiopathic scoliosis in females."

Hidewaki Nakagawa (Photo 12), Team Leader of the Laboratory for Genome Sequencing Analysis, received the Takeda Science Foundation, Visionary Research Fund Award for the study of plasma cell-free DNA sequencing of cancer patients.

Zijin Guo, Research Scientist in the Laboratory for Intestinal Ecosystem, received the Outstanding Poster Presentation Award for his presentation, "Establishment of a new allergy mouse model without using adjuvant" at the 12th European Academy of Allergy and Clinical Immunology (EAACI) Immunology Winter School held in Poiana Brasov, Romania.

Yoji Ogura, Visiting Scientist in the Laboratory for Bone and Joint Diseases received the Best Presentation Award at the 29th Annual Research Meeting of the Japanese Orthopaedic Association. His presentation "GWAS Analyses of Adolescent Idiopathic Scoliosis: Identification of a new genetic locus" was selected out of 852 presentations.

Rei Uematsu, Graduate Student in the Laboratory for Metabolomics, received the Young Scientist Award at the 13th Pharma-Bioforum of the Pharmaceutical Society of Japan for his oral presentation, "Eosinophils control the resolution of inflammation trough the 12/15-lipoxygenase pathway."

Kentaro Inoue, Postdoctoral Researcher in the Laboratory for Integrated Cellular Systems, received the Excellent Poster Award for his poster presentation entitled "Mathematical modeling to reveal switch-like and oscillatory activation of NF-κB transcription factor" at IIBMP (Informatics in Biology, Medicine and Pharmacology) 2014.

Shinnosuke Matsueda, Graduate Student in the Laboratory for Metabolomics, received the Young Scientist Award at the 87th Annual Meeting of the Japanese Biochemical Society. He was selected for his presentation "Analysis of the mechanism that regulates dynamics of eosinophil involved in the resolution of acute inflammation".

Takaharu Sasaki, Junior Research Associate in the Laboratory for Immune Cell Systems, received the Noyori Prize for poster at Noyori School 2014 for his presentation "Involvement of secondary lymphoid tissues in the induction of obesity".

RIKEN International Program Associate (IPA)

IMS accepted four international students as RIKEN International Program Associates (IPA). Under this IPA program, IMS lab heads host international students from collaborating graduate schools and supervise their Ph.D. program as Joint Supervisors. The students receive a daily living allowance and housing costs for up to a maximum of three years.

The IPA students who studied at IMS in 2014 were

Mohamed El Sherif Gadelhaq Badr (Tokyo Medical and Dental University) from Egypt studied in the Laboratory for Cell Signaling.
Mei Suen Kong (Universiti Sains Malaysia, Malaysia) studied in the Laboratory for Cell Signaling.
Kan Kaneko (University of Otago, New Zealand) studied in the Laboratory for Vaccine Design.
Evangelia Eirini Tsermpini (University of Patras, Greece) studied in the Laboratory for International Alliance on Genomic Research.
Chanyoung Shin (Tokyo Institute of Technology) from Korea studied in the Laboratory for Inflammatory Regulation.

RIKEN Foreign Postdoctoral Researcher (FPR) Program

The RIKEN Foreign Postdoctoral Researcher (FPR) program offers aspiring young foreign researchers with creative ideas and who show promise of becoming internationally active in the future the opportunity to pursue innovative research at RIKEN under the direction of a RIKEN laboratory head. The FPR Program is one of RIKEN's initiatives to open up its facilities and resources to the forefront of global science and technology.

In 2014, two young researchers studied at IMS as RIKEN FPRs.

Wooseok Seo studied in the Laboratory for Transcriptional Regulation.
Michelle Kendle Maslowski studied in the Laboratory for Intestinal Ecosystem.

RIKEN Junior Research Associate (JRA) Program

The Junior Research Associate Program was launched in 1996 to encourage young scientists with fresh ideas and youthful enthusiasm to collaborate with, and learn from, senior scientists with years of experience. This program provides part-time positions at RIKEN for young researchers enrolled in university Ph.D. programs. The JRA program serves the dual purpose of fostering the development of these young scientists while also energizing RIKEN with their innovative thinking.

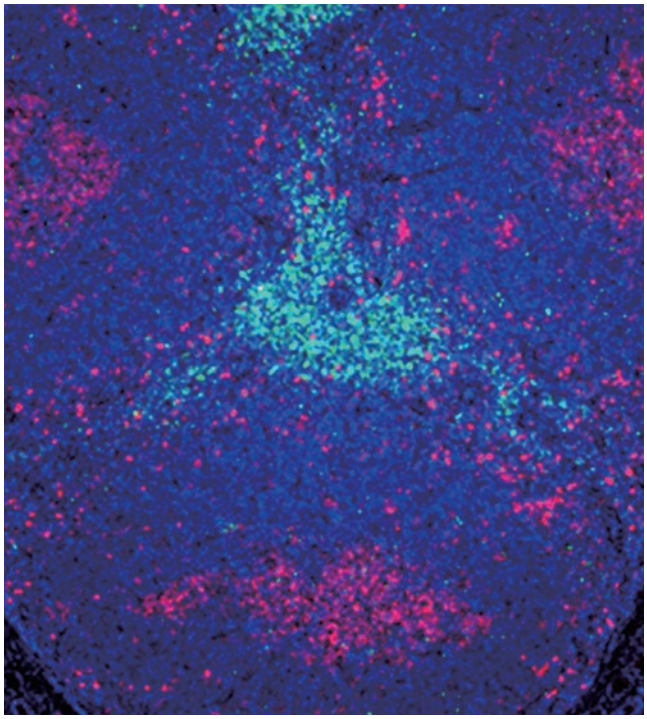
This year, 16 JRA students studied in IMS.

Hisashi Wada (Laboratory for Transcriptional Regulation)
Ryuichi Murakami (Laboratory for Immune Homeostasis)
Yujiro Yamamoto (Laboratory for Genome Sequencing Analysis)
Akemi Fujiwara (Laboratory for Intestinal Ecosystem)
Yusuke Sato (Laboratory for Immunotherapy)
Tomohiro Miyai (Laboratory for Immune Cell Systems)
Rintaro Ono (Laboratory for Human Disease Models)
Hirotsugu Oda (Laboratory for Integrative Genomics)
Yuki Furuichi (Laboratory for Skin Homeostasis)
Tadashi Takeuchi (Laboratory for Intestinal Ecosystem)
Rumiko Ono (Laboratory for Inflammatory Regulation)
Takaharu Sasaki (Laboratory for Immune Cell Systems)
Ryohei Aoyagi (Laboratory for Metabolomics)
Kensuke Yamaguchi (Laboratory for Autoimmune Diseases)
Yuma Sakamoto (Laboratory for Bone and Joint Diseases)
Atsushi Ono (Laboratory for Digestive Diseases)

RIKEN Special Postdoctoral Researcher (SPDR) Program

RIKEN's Special Postdoctoral Researcher Program was instituted to provide young and creative scientists the opportunity to be involved in autonomous and independent research in line with RIKEN objectives and research fields. The positions are competitive, but if selected, researchers receive salaries and research budgets (1million yen) from RIKEN and they are able to conduct their research at one of its laboratories.

This year, 4 postdocs conducted their research at IMS through the SPDR program.
Guillermo Juan Betancur Medina (Laboratory for Developmental Genetics)
Saya Moriyama (Laboratory for Tissue Dynamics)
Takeshi Tanoue (Laboratory for Gut Homeostasis)
Jun Miyata (Laboratory for Metabolomics)



Part 2

Research Projects

Expanding the immune system's memory

A newly identified subpopulation of innate immune cells can be 'primed' to provide a rapid response against the emergence of future threats, including tumor growth

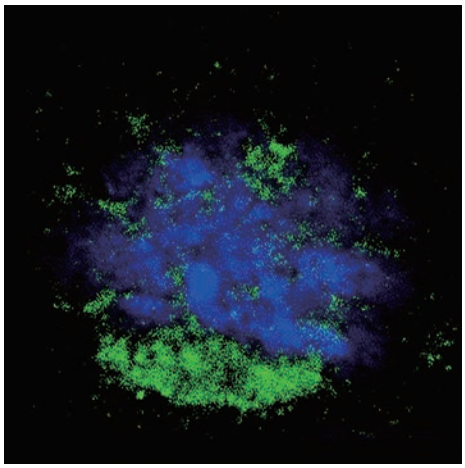


Figure: Fluorescence microscopy image showing KLRG1 (green) on the surface of an NKT cell (nucleus stained blue).

The adaptive immune system has the ability to 'remember' a given pathogen or cancer cell by producing memory T cells that can mount a rapid counterattack against future threats. The innate immune system, on the other hand, is seen as more of a blunt instrument, only capable of launching a broad defensive response against potential invaders. A research team led by Shin-ichiro Fujii and colleagues from the RIKEN Center for Integrative Medical Sciences has now identified a population of innate immune cells that display the attributes of memory cells and which may help keep tumor growth at bay.

Natural killer T (NKT) cells are recognized components of the innate immune system that are involved in the rapid response to virally infected cells and tumor formation. Recent studies, however, have hinted that after infection, some of these cells have many of the characteristics of memory cells. Spurred by clinical observations during NKT-cell therapy, Fujii's team began investigating a subtype called invariant natural killer T (iNKT) cells.

"We have found that lung cancer patients treated with dendritic cells and a lipid called α-galactosylceramide, which together modulate iNKT-cell activity, showed a longer median survival time, but we did not know the mechanism," says Fujii. Their research revealed that this activation specifically promotes the proliferation of iNKT cells expressing a particular surface protein known as killer cell lectin-like receptor subfamily G, member 1 (KLRG1), which is generally found on the surface of NK cells, adaptive immune cells, and memory T cells (Figure).

Examining the memory properties of these iNKT cells, Fujii's group learned that mice treated with dendritic cells and α-galactosylceramide maintain reservoirs of KLRG1-expressing iNKT cells in their lungs for up to nine months after treatment. Like memory T cells, these innate cells remain poised for a quick response and generated a vigorous immune reaction against a second dose of α-galactosylceramide injected several weeks or even months after the initial treatment. Additional experiments showed that the KLRG1-expressing iNKT cells produced by mice inoculated with dendritic cells and α-galactosylceramide could sharply reduce metastatic growth after injection with melanoma cells.

These findings reveal an additional layer of complexity for the innate immune system. "We have determined that the innate immune system can undergo a memory response," says Fujii. His group is now exploring whether the same memory-cell population can be identified and selectively stimulated in humans, offering a potential means for bolstering the protective response against cancer and other diseases.

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Original paper
Shimizu, K., Sato, Y., Shinga, J., Watanabe, T., Endo, T., Asakura, M., Yamasaki, S., Kawahara, K., Kinjo, Y., Kitamura, H., Watarai H., Ishii Y., Tsuji M., Taniguchi M., Ohara O., Fujii S. KLRG+ invariant natural killer T cells are long-lived effectors. ***Proc Natl Acad Sci U S A*** 111, 12474–12479 (2014).

Finding the roots of a spinal condition

A comprehensive genomic survey reveals six chromosomal regions containing risk factors for a spinal disorder particularly common in Japan

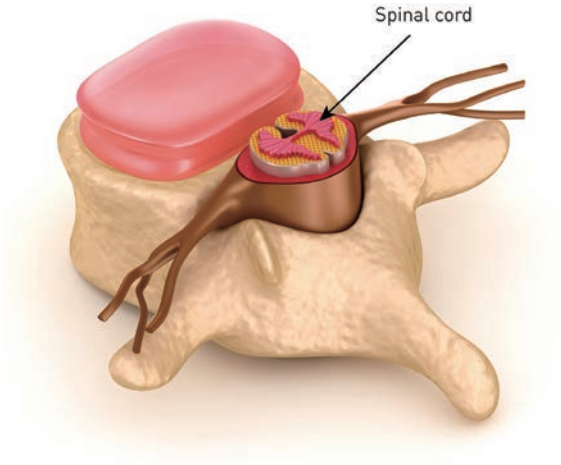

Figure: The spinal cord runs through the spinal canal, which is protected by soft tissue.

The spinal cord runs through a canal in the vertebrae that is lined with soft protective tissues. In patients with a condition known as ossification of the posterior longitudinal ligament of the spine (OPLL), bone begins to form within one of these tissues. The resulting narrowing of the spinal canal compresses the spinal cord, inflicting motor weakness, numbness and pain.

A large consortium of researchers headed by Shiro Ikegawa from the RIKEN Center for Integrative Medical Sciences has now uncovered a set of genetic factors that may contribute to the onset of this condition. Although the core causes of OPLL remain poorly understood, several indicators point to a strong hereditary component—including an unusually high prevalence among East Asians. "OPLL is one of the most common musculoskeletal diseases among middle-aged and elderly people in Asia, and affects more than two percent of the Japanese population," says Ikegawa.

As previous genetic studies on OPLL yielded ambiguous and contradictory data, Ikegawa teamed up with dozens of colleagues to search for additional risk factors across an unprecedented number of Japanese patients. The team combed through genomic data from more than 1,600 individuals suffering from OPLL and more than 13,000 healthy control subjects looking for DNA sequence variations with a statistically significant association with susceptibility to this disease. The results revealed six sites in the genome that appear to contain genetic risk factors, one of which had also been flagged in a previous genetic linkage analysis of OPLL.

Drilling deeper into these chromosomal segments, Ikegawa and his colleagues identified nearly a dozen candidate disease-associated genes, several of which exhibit suggestive functional links to OPLL. For example, the gene *RSPO2* encodes the R-spondin 2 protein, which helps to modulate the development and activity of bone-forming osteoblast cells. Another candidate gene, *RSPH9*, produces a protein that forms hair-like cellular structures known as cilia that play a prominent role in proper skeletal formation. Both of these genes fit into pathways that have been previously connected, either directly or indirectly, to other skeletal disorders. Other candidate genes serve more general cellular roles or perform unknown functions.

These results were obtained through independent analyses of two separate cohorts of subjects, and the resulting statistical significance gives Ikegawa great confidence in the importance of the findings. "We have found the first set of OPLL genetic loci," he says. "We now plan to identify the susceptibility genes within these loci and clarify the pathological mechanism of OPLL through functional studies."

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http://www.riken.jp/en/research/rikenresearch/highlights/7944/

Original paper
Nakajima, M., Takahashi, A., Tsuji, T., Karasugi, T., Baba, H., Uchida, K., Kawabata, S., Okawa, A., Shindo, S., Takeuchi, K., Taniguchi Y, Maeda S, Kashii M, Seichi A, Nakajima H, Kawaguchi Y, Fujibayashi S, Takahata M, Tanaka T, Watanabe K, Kida K, Kanchiku T, Ito Z, Mori K, Kaito T, Kobayashi S, Yamada K, Takahashi M, Chiba K, Matsumoto M, Furukawa K, Kubo M, Toyama Y. Genetic Study Group of Investigation Committee on Ossification of the Spinal Ligaments, Ikegawa S. A genome-wide association study identifies susceptibility loci for ossification of the posterior longitudinal ligament of the spine. ***Nat Genet*** 46, 1012–1016 (2014).

Gene regulation turned upside down

DNA binding by complexes that regulate developmental gene expression occurs in the reverse order to that expected

Figure: Polycomb-group proteins bind to DNA and repress expression of developmental genes.

Molecular modification of DNA and the histone proteins it is bound to is one of the key mechanisms responsible for regulating gene expression. Polycomb-group proteins play an important role in some of these modification processes, but the molecular mechanisms by which they bind to DNA and exert their effects have been poorly understood.

Takashi Kondo and colleagues from the Laboratory of Developmental Genetics at the RIKEN Center for Integrative Medical Sciences, in collaboration with researchers from the University of Oxford in the United Kingdom, have now shown that modifications involving Polycomb proteins occur in a manner that contradicts existing models.

Polycomb-group proteins are found in two complexes, known as PRC1 and PRC2, and there are two forms of PRC1—the canonical and variant forms. Molecular modifications involving these complexes play crucial roles in gene regulation, cellular differentiation and development. "Polycomb-group proteins repress gene transcription by binding to DNA," says Kondo. "This is the main system that regulates transcription of developmental genes, and Polycomb systems are also related to some cancers. Understanding their mechanism of action is therefore biologically very significant."

PRC1 and PRC2 complexes always work together, but exactly how has remained unclear. For some time, it has been thought that PRC2 must first bind to the DNA, which then allows binding or 'recruitment' of PRC1. However, as recent evidence suggests that this might not be the case, Kondo and his colleagues developed a new approach to investigate.

The researchers inserted human DNA into mouse embryonic stem cells to cause the PRC1 complex to bind at specific sites. They found that the variant form of PRC1, but not the canonical form, was able to recruit PRC2 to the DNA—the reverse of the long-accepted mechanism of PRC2 binding prior to recruiting PRC1.

This process is dependent on a component of the PRC1 complex called KDM2B, and deleting part of this protein prevented PRC1 from binding to DNA in cells. In live mice, genetically disrupting the function of KDM2B, thereby preventing PRC1 binding and PRC2 recruitment, had serious consequences: complete loss of KDM2B function was lethal before birth, while partial loss caused abnormalities in the skeleton due to incorrect development.

"Our findings indicate that the Polycomb regulatory mechanisms possibly rely on the activity of variant PRC1 rather than PRC2 or canonical PRC1," explains Kondo. "This means that studies on variant PRC1 may be more fruitful for investigating the mechanisms of developmental regulation and cancer development."

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http://www.riken.jp/en/research/rikenresearch/highlights/7821/

Original paper
Blackledge, NP., Farcas, AM., Kondo, T., King, HW., McGouran, JF., Hanssen, LLP., Ito, S., Cooper, S., Kondo, K., Koseki, Y., Ishikura, T., Long, HK., Sheahan TW., Brockdorff N., Kessler BM., Koseki H., Klose RJ. Variant PRC1 complex-dependent H2A ubiquitylation drives PRC2 recruitment and polycomb domain formation. ***Cell*** 157, 1445–1459 (2014)

A new target for allergy therapies

Immune cells called basophils are found to be key drivers of allergy-induced lung inflammation

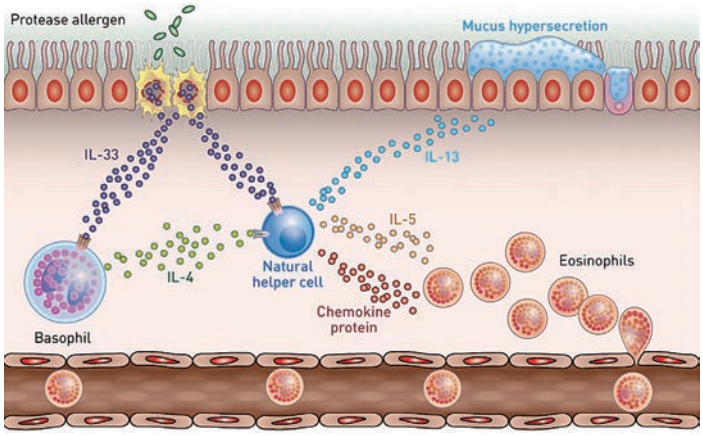

Figure: Lung inflammation that causes asthma is linked to the secretion of interleukin-4 (IL-4) from immune cells called basophils.

Many particles and molecules in the environment can trigger allergic asthma in susceptible individuals. The allergic response to some of these allergens results in lung inflammation that can lead to a narrowing of the airways and even severe respiratory difficulty. A research team led by Masato Kubo from the RIKEN Center for Integrative Medical Sciences has now identified that a type of immune cell called a basophil is responsible for initiating a cascade of events that leads to inflammation of the lung in mice after exposure to plant- and dust-mite-derived allergens.

Protein-chewing enzymes known as proteases that are derived from dust mites and plants can begin to break down cells in the outer layer of the lung, causing damage that can initiate a local inflammatory reaction. While investigating the triggers of this inflammation, the researchers found that these protease allergens induced lung inflammation in normal mice with a complete immune system, but not in mice genetically engineered to lack basophils.

Immune cells, including basophils, secrete the cytokine interleukin-4 (IL-4), which is known to play a role in the induction of asthma. The extent to which basophil-derived IL-4 is involved in the induction of lung inflammation, however, was unknown. Kubo and his colleagues showed that proteases could increase the expression of IL-4 in basophils but not in other types of immune cells. In mice whose basophils were unable to produce IL-4, protease treatment did not cause lung inflammation, suggesting that basophil-derived IL-4 could be the main driver of protease-allergen-induced asthma in mice—a finding that could also extend to humans.

Natural helper (NH) cells are another type of immune cell that is known to play a role in the body's response to allergens. The researchers showed that NH cells express the receptor for IL-4, and that NH cells treated with IL-4 increase their expression of cytokines such as IL-5 and IL-13, which are also known to be involved in asthma induction. IL-4-treated NH cells also demonstrate a rise in expression of various chemokine proteins known to attract large numbers of eosinophil immune cells to the lung (Figure). This influx of eosinophils triggers inflammation and narrowing of the airway, which leads to asthmatic symptoms such as wheezing.

The findings suggest that treatments that reduce the numbers of basophils, or that prevent the production of IL-4 by basophils, could be promising for the management of lung inflammation and asthma caused by protease allergens.

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http://www.riken.jp/en/research/rikenresearch/highlights/7815/

Original paper
Motomura, Y., Morita, H., Moro, K., Nakae, S., Artis, D., Endo, TA., Kuroki, Y., Ohara, O., Koyasu, S., Kubo, M. Basophil-derived interleukin-4 controls the function of natural helper cells, a member of ILC2s, in lung inflammation. ***Immunity*** 40, 758–771 (2014)

Twenty-three genetic variants identified that confer susceptibly to prostate cancer

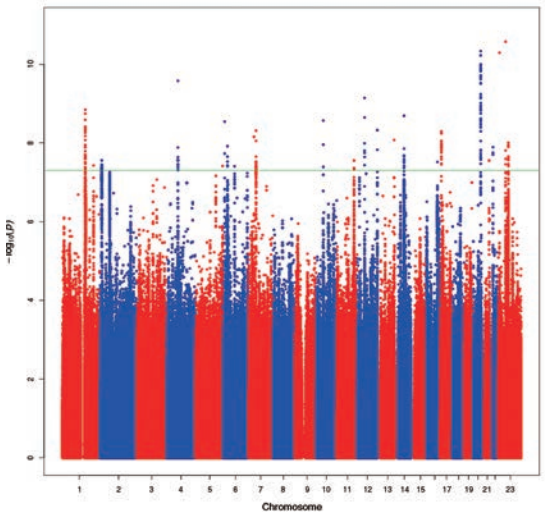

Figure: Manhattan plot of the results from the multi-ancestry meta-analysis of overall prostate cancer risk. The 23 peaks above the green line ($P=5\times10^{-8}$) indicate an association with prostate cancer. The 77 already reported GWAS SNPs associated with prostate cancer were omitted.

A study conducted by Hidewaki Nakagawa of RIKEN Center for Integrative Medicine (IMS), in a very large international collaboration effort including with the University of Southern California, The Institute of Cancer Research and the University of Cambridge, has revealed 23 new human genetic susceptibility loci indicating risk for prostate cancer.

Prostate cancer is one of the most common malignancies in males throughout the world. Asian populations have the lowest incidence and mortality rate of prostate cancer in the world, but its incidence is rapidly increasing in Japan and other Asian countries. According to National Cancer Center of Japan, the age-adjusted incidence of prostate cancer has increased 8 fold, from 7.1 out of 100 thousand people in 1975 to 56.0 in 2010. Although the mechanisms by which prostate cancer develops and progresses are not clear, there is considerable evidence that genetic factors are important in its etiology.

In 2010 and 2012, Nakagawa's team reported 9 genetic variants associated with prostate cancer susceptibility in a Japanese population using a genome-wide association study (GWAS).

To identify additional prostate cancer susceptibility loci, the international research group analyzed genetic data of 87,040 men from European, African, Japanese and Latino ancestry. This is the largest study of its kind and is the first that combines multiple studies across different ethnic populations.

Past GWAS have identified 77 variants associated with prostate cancer risk. The 23 new variants identified in the current study bring the total number of common genetic variants linked to prostate cancer to 100. The new study shows that for European men assessed for the 100 common variants, the 10% at highest risk are 2.9 times more likely than the average person to develop prostate cancer, while the top 1% are 5.7 times more likely to develop the disease. These findings demonstrate the importance of conducting large-scale genetic studies in diverse populations for the discovery of new risk loci that continue to provide new insights into disease mechanisms for complex traits.

Original paper
Al Olama AA, Kote-Jarai Z, Berndt SI, Conti DV, Schumacher F, Han Y, Benlloch S, Hazelett DJ, Wang Z, Saunders E, Leongamornlert D, Lindstrom S, Jugurnauth-Little S, Dadaev T, Tymrakiewicz M, Stram DO, Rand K, Wan P, Stram A, Sheng X, Pooler LC, Park K, Xia L, Tyrer J, Kolonel LN, Le Marchand L, Hoover RN, Machiela MJ, Yeager M, Burdette L, Chung CC, Hutchinson A, Yu K, Goh C, Ahmed M, Govindasami K, Guy M, Tammela TL, Auvinen A, Wahlfors T, Schleutker J, Visakorpi T, Leinonen KA, Xu J, Aly M, Donovan J, Travis RC, Key TJ, Siddiq A, Canzian F, Khaw KT, Takahashi A, Kubo M, Pharoah P, Pashayan N, Weischer M, Nordestgaard BG, Nielsen SF, Klarskov P, Røder MA, Iversen P, Thibodeau SN, McDonnell SK, Schaid DJ, Stanford JL, Kolb S, Holt S, Knudsen B, Coll AH, Gapstur SM, Diver WR, Stevens VL, Maier C, Luedeke M, Herkommer K, Rinckleb AE, Strom SS, Pettaway C, Yeboah ED, Tettey Y, Biritwum RB, Adjei AA, Tay E, Truelove A, Niwa S, Chokkalingam AP, Cannon-Albright L, Cybulski C, Wokołorczyk D, Kluźniak W, Park J, Sellers T, Lin HY, Isaacs WB, Partin AW, Brenner H, Dieffenbach AK, Stegmaier C, Chen C, Giovannucci EL, Ma J, Stampfer M, Penney KL, Mucci L, John EM, Ingles SA, Kittles RA, Murphy AB, Pandha H, Michael A, Kierzek AM, Blot W, Signorello LB, Zheng W, Albanes D, Virtamo J, Weinstein S, Nemesure B, Carpten J, Leske C, Wu SY, Hennis A, Kibel AS, Rybicki BA, Neslund-Dudas C, Hsing AW, Chu L, Goodman PJ, Klein EA, Zheng SL, Batra J, Clements J, Spurdle A, Teixeira MR, Paulo P, Maia S, Slavov C, Kaneva R, Mitev V, Witte JS, Casey G, Gillanders EM, Seminara D, Riboli E, Hamdy FC, Coetzee GA, Li Q, Freedman ML, Hunter DJ, Muir K, Gronberg H, Neal DE, Southey M, Giles GG, Severi G; Breast and Prostate Cancer Cohort Consortium (BPC3); PRACTICAL (Prostate Cancer Association Group to Investigate Cancer-Associated Alterations in the Genome) Consortium; COGS (Collaborative Oncological Gene-environment Study) Consortium; GAME-ON/ELLIPSE Consortium, Cook MB, Nakagawa H, Wiklund F, Kraft P, Chanock SJ, Henderson BE, Easton DF, Eeles RA, Haiman CA. A meta-analysis of 87,040 individuals identifies 23 new susceptibility loci for prostate cancer. ***Nat Genet*** 46, 1103–1109 (2014).

An all or nothing proposition

A feedback signaling system forms the foundation for a cellular on–off switch that regulates immune responses

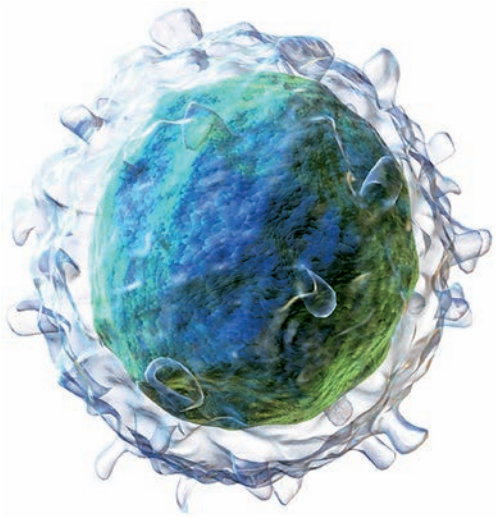

Figure: B cells (shown) are a type of lymphocyte that play a crucial role in the immune response.

The immune system contains a vast array of cell types and signaling pathways that help to regulate immune response. Among these cell types are B cells, which have the unique ability to bind to specific antigens due to a transmembrane receptor protein known as the B cell receptor (BCR) on the cell surface. Through a combination of biochemical and mathematical modeling experiments, a research team led by Mariko Okada-Hatakeyama from the RIKEN Center for Integrative Medical Sciences has now gained a deeper understanding of the interplay between BCRs and another key component of the immune response, the NF-κB protein complex.

When a BCR recognizes a potential threat, it sets into motion a series of cellular events that promote a broader immune response. A key step in this process occurs when BCR signaling causes NF-κB to migrate from the cytoplasm to the nucleus, where it directly binds and activates numerous target genes that stimulate B cell maturation and antibody production.

Okada-Hatakeyama and her colleagues uncovered evidence that BCR-stimulated NF-κB activation operates via a binary switch mechanism in which any signal that crosses a set threshold triggers strong, long-lasting activation. Initial cell culture experiments showed that the core machinery of this switch resides in the interaction between two proteins: TAK1 and IKKβ. BCR signaling is initially transmitted from TAK1 to IKKβ, which in turn activates NF-κB. However, the researchers also discovered a 'positive feedback' loop mediated by phosphorylation of CARMA1 at Ser578, wherein activated IKKβ gives a further boost to TAK1 activation and thereby increases NF-κB activity. Computational simulations supported this model and confirmed that interference with this feedback loop effectively kills the switch-like response.

This on–off signaling mechanism has important functional consequences. "All-or-none responses are very important because after an 'on' response it is very difficult to return to the basal state," says Okada-Hatakeyama. "This means that if a disease state develops, total recovery is very difficult." These findings could therefore prove helpful in understanding the role of NF-κB in certain cancers or inflammatory disorders. On the other hand, such signaling switches can also confer considerable stability to systems, eliminating the signaling 'noise' that can occur in more dynamic signaling networks.

The researchers now hope to explore the final impact of this switch mechanism at the level of NF-κB target gene activity. "We are also planning to rewire and modify this signaling network and see how NF-κB activation and B cell differentiation processes are changed in living systems," says Okada-Hatakeyama.

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Original paper
Shinohara, H., Behar, M., Inoue, K., Hiroshima, M., Yasuda, T., Nagashima, T., Kimura, S., Sanjo, H., Maeda, S., Yumoto, N., Ki, S., Akira, S., Sako, Y., Hoffmann, A., Kurosaki, T., Okada-Hatakeyama, M. Positive feedback within a kinase signaling complex functions as a switch mechanism for NF-κB activation. ***Science*** 344, 760–764 (2014)

Dying cells trigger immunity

The release of nucleic acids from dying cells induces the maturation of a type of immune cell that fights parasites and drives allergic reactions

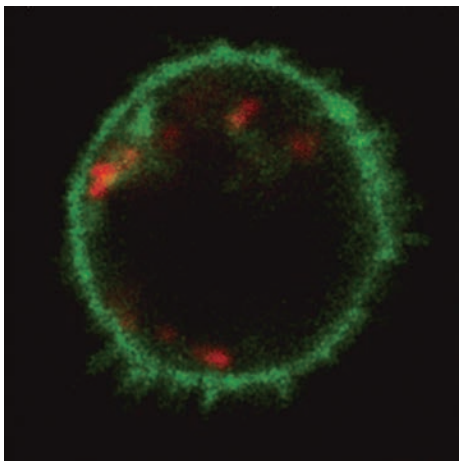

Figure: DNA (*red*) is taken up inside naive T cells (*green*), leading to their activation and differentiation into T helper type 2 (Th2) cells.

The immune system produces various types of immune cells—some are pre-programmed to target pathogens that the immune system has previously encountered, while others are 'naive' and retain the ability to mature or differentiate into specific cell types to target new invaders. Some of the triggers of this differentiation, however, remain poorly understood.

Takashi Saito, Takayuki Imanishi and colleagues from the Laboratory for Cell Signaling at the RIKEN Center for Integrative Medical Sciences have now led an international team of researchers that has found that nucleic acids, such as DNA and RNA, released from dying cells can trigger naive immune T cells to differentiate into T helper type 2 (Th2) cells.

The researchers performed a series of experiments where naive T cells were cultured with different kinds of nucleic acid species to induce T-cell activation. They found that certain classes of nucleic acids that tended to interact with other nucleic acids were more effective at activating T cells (Figure), suggesting that these structural interactions enhance nucleic acid stability and uptake. Nucleic acids bound to various antimicrobial peptides and proteins—typical of the nucleic acids released by dying cells—also tended to promote T-cell activation, indicating that activation occurs at sites of inflammation or infection.

"Nucleic acids have previously been shown to be recognized by innate immune cells that present antigens to stimulate T cells," explains Saito, "but this study clarifies that T cells are directly activated by the nucleic acids themselves."

In many types of immune cells, nucleic acids bind to a class of proteins called Toll-like receptors (TLRs), which sense pathogen-associated molecular patterns to initiate innate responses and help regulate T cell-mediated adaptive immune responses. The researchers were surprised to find that TLRs did not seem to play a role in nucleic-acid-driven T-cell differentiation.

Instead, the researchers found that exposure to nucleic acids induced in the naive T cells the expression of a transcription factor that is known to specifically drive Th2 maturation. This was evidenced by the secretion of proteins characteristic of Th2 cells when naive T cells were cultured with nucleic acids.

Th2 cells drive the immune response against parasitic worms and serve a key role in triggering allergic reactions. The findings therefore suggest that blocking the ability of nucleic acids to induce Th2 maturation could be a promising possible therapeutic approach to reducing the severity of allergies in humans.

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Original paper
Imanishi, T., Ishihara, C., Badr, MESG., Hashimoto-Tane, A., Kimura, Y., Kawai, T., Takeuchi, O., Ishii, KJ., Taniguchi, S., Noda, T., Hirano, H., Brombacher, F., Barber, GN., Akira, S., Saito, T. Nucleic acid sensing by T cells initiates Th2 cell differentiation. ***Nat Commun*** 5, 3566 (2014)

A gut feeling on bacterial diversity

The immune system interacts with microbes in the intestines to promote rich and balanced bacterial communities

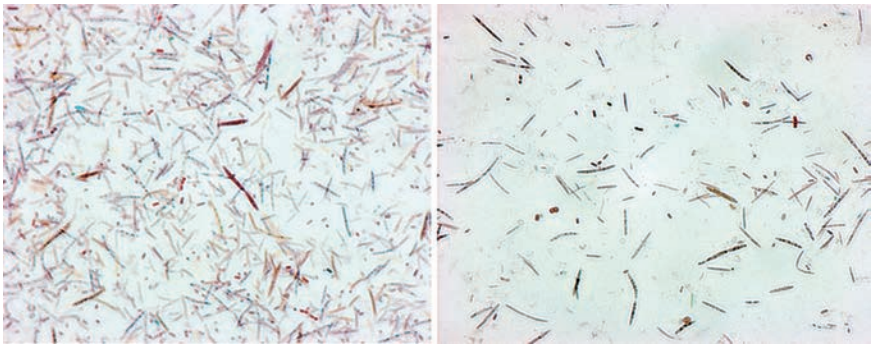

Figure: Gut bacteria from mice with rich (left) or poor (right) microbial communities.

The primary function of the human immune system is to fight off infections. Yet in the gut, the immune system can also nourish communities of symbiotic microbes that help maintain proper health. Just how the body does this is not fully understood. Researchers led by Sidonia Fagarasan from the Laboratory for Mucosal Immunity at the RIKEN Center for Integrative Medical Sciences have now demonstrated that regulatory T cells play a role in shaping the community of trillions of bacteria that inhabit the digestive tract.

"We found that the immune system is actively involved in the maintenance of rich and balanced bacterial communities in the gut," says Fagarasan. "Our study reveals a different picture of the immune system, one involving sophisticated, two-way interactions between bacteria and immune cells."

Fagarasan and her colleagues started by studying mice that were deficient in various cellular and structural components of the gut immune system. They showed that these mice had reduced bacterial diversity. When the missing components were added back incrementally, they discovered that regulatory T cells—a type of immune cell that helps keep immune responses in check—were needed to control levels of an antibody called immunoglobulin A, which is produced in mucosal linings where it helps select for 'good' bacteria.

The crosstalk between the immune system and bacteria seemed to go both ways; the presence of more diverse gut flora seemed to spur a positive feedback loop within the immune system. Fagarasan's team tested this by introducing gut microbes taken from different types of bacterial communities into mice born and bred in sterile incubators. They discovered that the immune system naturally responded to rich and balanced bacterial populations by inducing the proliferation of regulatory T cells and immunoglobulin A, which further supported a diversified microbiome. In contrast, less varied bacterial communities induced the proliferation of inflammatory T cells and the antibodies immunoglobulin G and E.

"This means that immune deficiencies, regardless of whether inherited, acquired by external interventions or simply brought on by aging, will have an impact on the bacterial communities, and likely reduce their diversity and ecologic stability," notes Fagarasan.

When it comes to therapeutics for gut-associated diseases, many drug developers are now focused on developing probiotics or fecal transplants to introduce desired bacteria. According to Fagarasan, however, therapies that modify the immune system might also be necessary. "We need to think about correcting not only the bacterial imbalance but also re-establishing immune homeostasis," she says.

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http://www.riken.jp/en/research/rikenresearch/highlights/7841/

Original paper
Kawamoto, S., Maruya, M., Kato, L. M., Suda, W., Atarashi, K., Doi, Y., Tsutsui, Y., Qin, H., Honda, K., Okada, T., Hattori M., Fagarasan S. Foxp3$^+$ T cells regulate immunoglobulin A selection and facilitate diversification of bacterial species responsible for immune homeostasis. ***Immunity*** 41, 152–165 (2014).

iPS Project

Induced pluripotent stem (iPS) cells possess tremendous therapeutic potential in the many areas, including regenerative medicine and immune therapy. We have begun an activity to apply iPS technology to both mouse and human immunology research and to development of therapeutics. On a collaborative basis with individual IMS research laboratories, the core facility for iPS research is engaged in developing efficient protocols to reprogram various types of lymphocytes into iPS cells as well as to induce differentiation of iPS cells into a variety of lymphoid lineage cells. This activity is partly supported by the Japan Science and Technology Agency (JST).

This year, the facility established iPS cells from human natural killer T (NKT) cells. Because of the potent anti-tumor activity of NKT cells, tumor immunotherapy using activated NKT cells has already been approved in Japan as Advanced Medicine, and clinical trials have started. NKT-cell-targeted therapy, however, is restricted to a minority of patients, because often cancer patients suffer from a decrease in both the number and functional potency of NKT cells. NKT cells were transduced with Yamanaka factors, after which they established clones that formed colonies with human embryonic stem cell-like morphology. When co-cultured with OP9/DLL1 cells, the NKT-iPS cells efficiently differentiated into NKT cells (iPS-NKT). Stimulation of these iPS-NKT cells with dendritic cells that had been pulsed with the NKT cell ligand α-galactosylceramide (DC/G) resulted in the production of IFNγ. The present study thus provides a novel method for cloning and expanding functional NKT cells, which can potentially be infused into autologous patients and applied for NKT cell-mediated tumor immunotherapy.

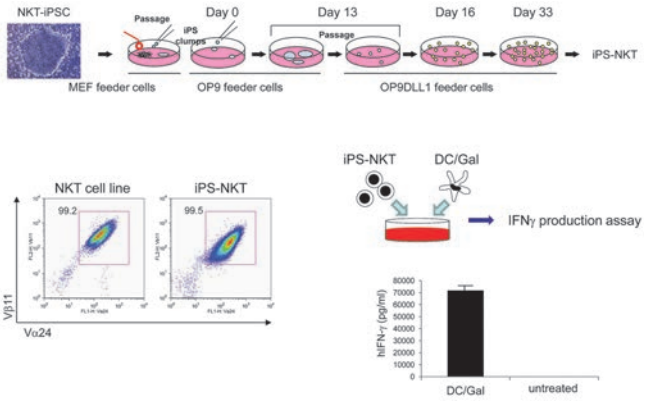


Figure: iPS based approach for regeneration of functional NKT (iPS-NKT) cells.
NKT derived iPSCs (NKT-iPSC) were separated into small clumps and plated onto OP9 feeder cells. On day 13, cells were transferred to co-culture with OP9-DLL1 feeder cells. On day 33, expression of the NKT cell specific TCR was confirmed by flow cytometry. These cells produced a substantial amount of INFγ upon DC/G stimulation.

Primary Immunodeficiency (PID) Project

As an important activity in former RCAI, we have constructed a network with pediatricians in Japan and are functioning as a hub of the network in the PID project. With the help of the Kazusa DNA research Institute and the Clinical PID Study Group, RCAI had established a clinical archive for PID patients in Japan, termed PIDJ. Based on the success of PIDJ, RCAI extended the PID network to include Asia and created a new database, Resource of Asian Primary Immunodeficiency Diseases (RAPID). Given the presence of an existing active network in Japan and taking the opportunity to merge with the strong human genetics teams coming from the former CGM, IMS has decided to continue focusing on PID. We continue to provide a genetic testing service for PID candidate genes in response to requests from pediatricians. This activity further strengthens the PID network and contributes a great deal to bridging gaps between clinical and basic immunologists.

In addition to this screen for causative genetic variation(s), IMS has started to apply the whole-exome sequencing (WES) approach with the goal of identifying responsible gene mutations in CVID (common variable immunodeficiency) patients. In the past year, we performed WES on samples of five CVID patients. After validating these results, we are now focusing on mutations in two genes in two different patients, and have moved forward to perform functional assays to verify the potential role of these genes in CVID. This approach includes analyses of gene functions in cell lines and in mouse models, and reconstitution of hematopoiesis in the humanized mouse system. We believe that the WES approach will identify novel CVID causal genes.

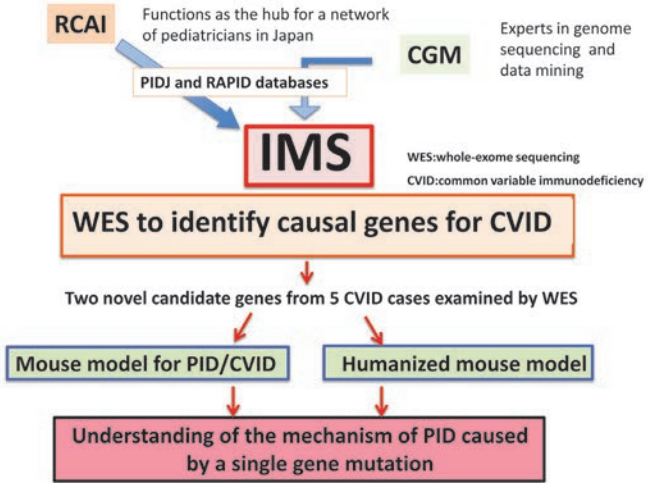


Figure: Strategy for the PID project at IMS
This figure shows a flowchart of how IMS will identify CVID causal gene(s) via a whole-exome sequencing (WES) approach.

Modeling Skin Diseases

The pathogenesis of human diseases is a highly complex process because of the complexity of not only the pathogenetic insults and homeostatic responses to them, but also of the structures of organs and tissues that include many different types of cells. To tackle such complexity, IMS has initiated center-wide projects to understand the pathogenesis of atopic dermatitis (AD), autoimmune diseases, primary immunodeficiency and others, in which multiple research groups work interactively and synergistically to achieve their common objectives. In each project, the first aim is to understand molecular and cellular networks underlying homeostasis of each organ/tissue.

In the atopic dermatitis project, Dr. Taylor's group has been developing an integrated database and sample-tracking system for the storage and distribution of massive and various types of experimental data including: flow cytometry data, immunohistochemistry images, skin water loss measurements, blood profiles, RNA-Seq data, etc. They have been working with colleagues in the experimental labs, Drs. Hisahiro Yoshida, Masato Kubo, Takaharu Okada and Masayuki Amagai, to better understand their data, and in the computational analysis and modeling labs led by Drs. Mariko Okada, Hiroaki Kitano, Osamu Ohara and Tatsushiko Tsunoda, for their analysis requirements and preferences for capturing the data in the database.

Drs. Yoshida and Kubo generated AD mouse models and, using their samples, Dr. Ohara's group has conducted transcriptome analysis. Drs. M. Okada and Kitano's groups perform data-driven prediction of the order of events occurring in AD progression. Dr. T. Okada investigates nerve fiber structure in the dermis and epidermis by whole-mount staining of mouse ear skin and confocal fluorescence microscopy. Drs. Amagai and Tsunoda have been working to connect the findings between mouse and human AD and discover the common mechanism of the disease onset.

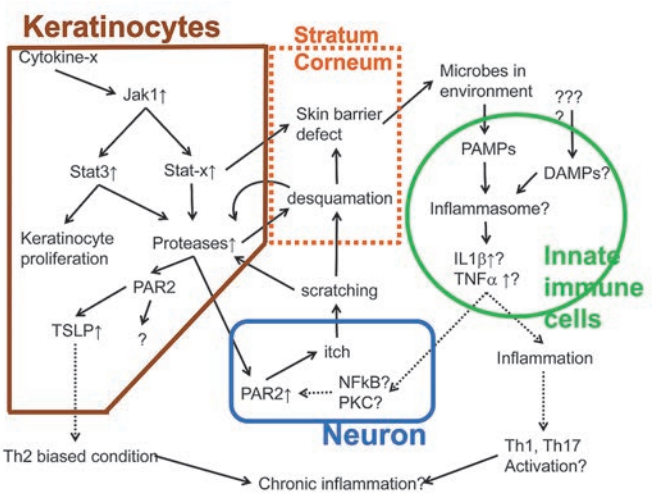
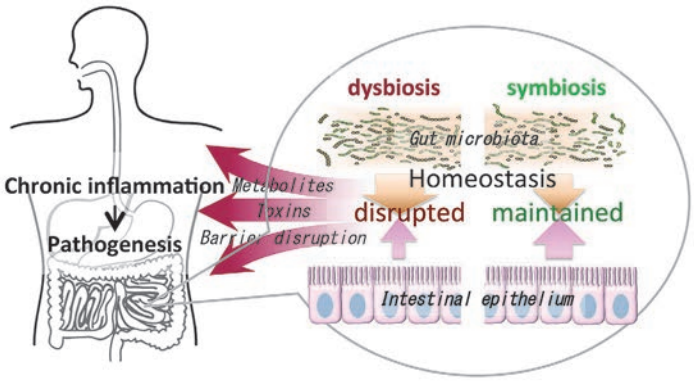


Figure: Molecular and cellular events preceding atopic dermatitis onset in mouse skin tissue
A genetic factor, in this case a point mutation in the Jak1 signal transduction molecule, induces protease overexpression in the epidermis which induces sequential events in various other cells in the skin tissue under the influence of environmental factors, ultimately resulting in atopic dermatitis.

Impact of Host-gut Microbiota Interactions on the Pathogenesis of Diabetes

Accumulating evidence from recent studies indicates that changes in gut microbiota composition and the loss of its diversity, a condition called dysbiosis, are not the consequence but rather the cause of various diseases including diabetes (Figure). As a Center project, we are using a comprehensive multiple omics approach to study the role of host-gut microbiota interactions in the pathogenesis of type 2 diabetes. The project is being done in collaboration with Professors Takashi Kadowaki and Tsutomu Yamazaki from the University of Tokyo Hospital and Professor Masahira Hattori from the Center for Omics and Bioinformatics, the University of Tokyo. The core participating Laboratories from RIKEN IMS are Metabolic Homeostasis, Intestinal Ecosystem, Metabolomics, and Integrated Bioinformatics. Fecal metagenomic, metatranscriptomic and metabolomic data will be obtained from diabetic patients and healthy counterparts. Metabolomic data will also be obtained from blood and urine samples. In addition, exome sequencing and SNP analysis of disease susceptibility genes will be performed. The goal is to identify disease risk factors, such as the presence or absence of certain bacteria and/or their metabolites, by analyzing the meta data derived from the comprehensive multiple omics approach combined with conventional clinical and genetic datasets.



Schematic view of symbiosis and dysbiosis in disease pathogenesis
In healthy individuals, the gut microbiota is robust and resistant to perturbations, for example caused by antibiotics or bacterial/viral infections, and its composition is maintained within a normal range to sustain homeostasis, a condition of symbiosis. However, in susceptible individuals, genetic predispositions may make it difficult to maintain homeostasis once the microbial composition or its diversity is perturbed and then the change becomes irreversible. The resultant abnormal microbiota, a condition of dysbiosis, can have a causative role in diseases by evoking a chronic inflammatory state.

Linkage to RIKEN Program for Drug Discovery and Medical Technology Platforms (DMP)

IMS collaborates with DMP to develop innovative new pharmaceuticals and medical technologies by facilitating the transfer of basic research within the institute.

The DMP was founded in RIKEN in 2010 in order to support all phases of development of new therapeutics, from the discovery of promising targets to the identification of potential lead compounds, such as small molecules and antibodies, and the acquisition of intellectual property rights to drugs and technologies that can then be brought to the development phase. The implementation of drug discovery requires a different technology, thus DMP established several medical technology platforms that promote research and development. IMS contributes to this effort in several ways, including by setting up a facility for development of antibody drugs.

IMS now has five programs in association with DMP, including Artificial adjuvant vector cells (Shin-ichiro Fujii), Leukemia treatment drugs targeting leukemic stem cells (Fumihiko Ishikawa), Cancer treatment with NKT cells (Haruhiko Koseki), Drugs for allergic diseases (Masaru Taniguchi), and a Mucosal vaccine delivery system (Hiroshi Ohno). The Artificial adjuvant vector cells project for cancer therapy is now at the preclinical phase of drug development.

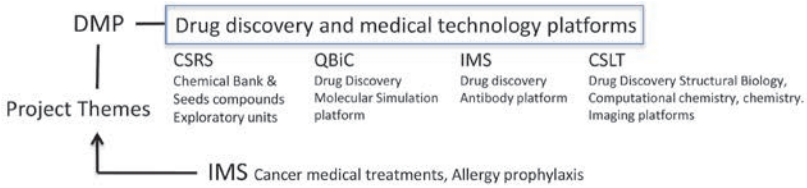


Figure: IMS links to the DMP project

Humanized Mouse Research

Through creation of humanized mouse models, we have been investigating normal and diseased human hematopoiesis. In 2014, we established an NSG xenotransplant model for mixed lineage leukaemia (*MLL*)-rearranged acute lymphoblastic leukemia in collaboration with the Japan Pediatric Leukemia Study Group (Aoki et al., Blood, 2015). Considering the fact that MLL leukemia is one of the most intractable of pediatric hematologic malignancies, we first aimed to identify leukemia-initiating-cells (LICs) by transplanting patient samples into NSG mice. *MLL* rearrangement occurs through translocations involving the *MLL* gene on chromosome 11 (11q23) and other chromosomes, generating MLL fusion proteins with multiple partners such as AF4 and AF9. We found that $CD34^+CD38^+$ and $CD34^-$ cells initiated leukemia in cases with the *MLL-AF4* translocation and that $CD34^-$ cells initiated leukemia in cases with the *MLL-AF9* translocation. In both translocations, $CD34^+CD38^-CD19^-CD33^-$ cells appeared to be normal HSC/HPCs, as evidenced by their ability to differentiate into normal mature $CD10^-CD20^+$ B cells in the spleen, $CD15^+$ neutrophils in the bone marrow, and $CD4^+CD8^+$ T cells in the thymus of the NSG recipients. Transcriptome analyses identified the gene signatures of normal HSCs and LICs in *MLL*-rearranged leukemia. Genes that are differentially expressed between normal and malignant stem cells could be valuable therapeutic targets. Among plasma membrane molecules, we found that CD9, CD32, CD24, and CD180 are expressed by LICs, but not by normal HSC/HPCs from *MLL*-rearranged leukemia patients.

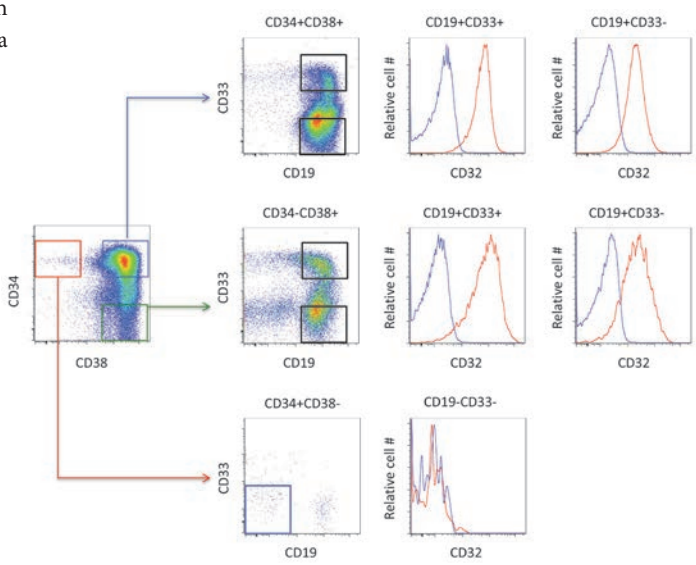


Figure: Differential expression of CD32 by normal HSCs and leukemia-initiating-cells with the *MLL* rearrangement

In a case with the *MLL-AF4* translocation, we found heterogeneous cell fractions, $CD34^+CD38^+$ and $CD34^-$ LICs as well as $CD34^+CD38^-$ HSCs. $CD34^+CD38^+$ and $CD34^-$ LICs were further divided into $CD33^+$ and $CD33^-$ fractions. All four fractions $CD34^+CD38^+CD19^+CD33^-$ cells, $CD34^+CD38^+CD19^+CD33^+$ cells, $CD34^-CD19^+C33^-$ cells, and $CD34^-CD19^+CD33^+$ cells express CD32. In contrast, $CD34^+CD38^-CD19^-CD33^-$ HSCs are negative for the expression of CD32. Therefore, CD32 could be a therapeutic target for *MLL*-rearranged leukemia.

NKT Project

NKT cells are known to enhance immune responses. The medical innovation groups in IMS have launched projects aimed at application of NKT cell therapy to cancer and allergic disease as follows.

First, based on our previous success using dendritic cells (DCs) loaded with the NKT ligand α-galactosylceramide (α-GalCer) for treatment of advanced lung cancer, we have begun a collaboration with the National Hospital Organization of a randomized phase IIa trial in early stage lung cancer and also a collaboration with Chiba University for a phase IIa trial for head and neck cancer. Second, based on our previous approaches in which we generated human iPS-derived T cells as well as mouse iPS-NKT cells, we successfully established and characterized human iPS-NKT cells during this fiscal year. This IMS iPS project on NKT cell-targeted therapy has been accepted as a clinical application research project, and is supported by the research center network for realization of regenerative medicine. Third, we established artificial adjuvant vector cells against cancer that are composed of tumor mRNA and α-GalCer, leading to activation of both innate and adaptive immunity. We have been working on preclinical studies through discussions with the Pharmaceuticals and Medical Devices Agency (PMDA). This project has now been supported by the translational research network program. Fourth, we have developed a new chemical compound that can selectively be delivered to B cells, resulting in the preferential suppression of IgE production. This drug could be applicable for asthma, pollinosis or food allergy. The project is supported by the RIKEN Drug Discovery and Medical Technology Platforms and the Scientific Research Fund from Health and Welfare.

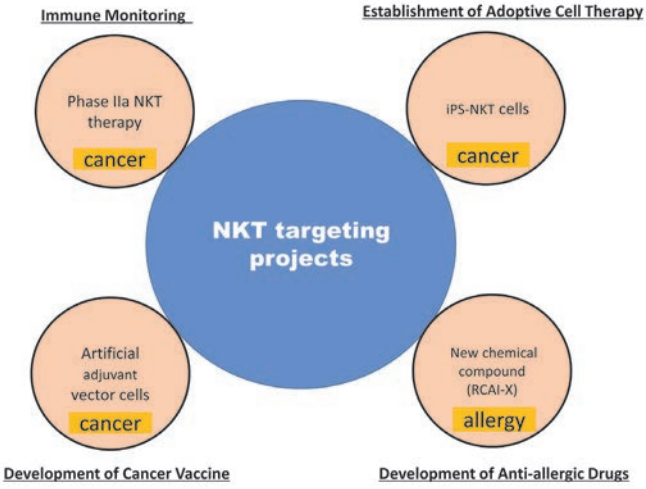


Figure: NKT projects in IMS as translational research
Four NKT projects have been launched in IMS as translational research projects. Three projects are for cancer treatment and one is for treatment of allergic diseases.

Allergy Project

We showed that delivery of α-galactosylceramide (α-GalCer), a representative ligand for invariant natural killer T (NKT) cells, to subsets of splenic B220-positive cells, including IgE-expressing B cells, led to IL-21 expression by iNKT cells and resulted in isotype-specific suppression of IgE responses. To develop this activity into an IgE-suppressive drug, we screened α-GalCer analogue compounds by assessing IL-21 expression in NKT cells derived from mice injected the liposomal compounds intravenously. Three compounds, RCAI-X, Y and Z were selected. General toxicology and genetic toxicology testing were carried out in mice with each liposome formulation. All liposomal compounds were well tolerated when given up to three times/week at intravenous injection doses up to 5 mg/kg over two weeks (6 total doses). During these pharmacological studies, we found that liposomal RCAI-X, but not the other compounds, enhanced IL-21 expression by NKT cells and suppressed the *in vivo* secondary IgE antibody response. Based on these results, we decided to develop the RCAI-X compound as the first candidate IgE-suppressive drug. In 2014, we were scheduled to complete the development of the RCAI-X manufacturing process with a collaborative company, Kaken Pharmaceutical Co. Ltd., but failed to do so due because too many steps were required for the synthesis of the RCAI-X compound. To recover from this developmental delay, we started to re-screen RCAI compounds, with the hope of finding one whose synthetic process would be much simpler than that of RCAI-X. In parallel, we are attempting to establish a new RCAI-X synthesis process. This project is supported by the RIKEN Drug Discovery and Medical Technology Platforms and the Scientific Research Fund from Health and Welfare.

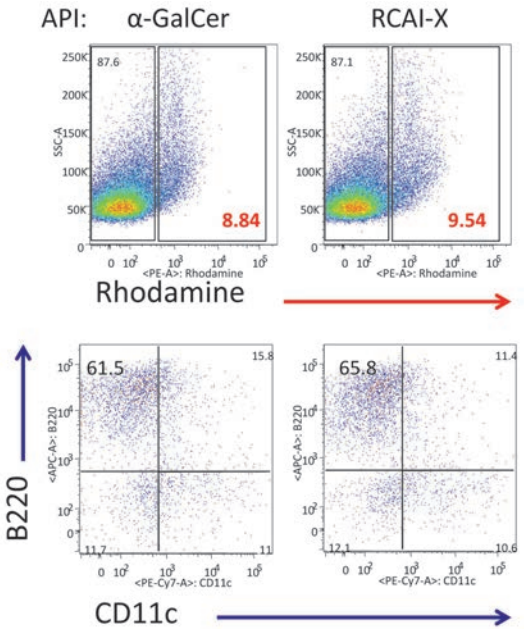


Figure: Comparison of the incorporation efficacy of α-GalCer and RCAI-X liposomes into splenic B220-positive cells.

Influenza Project

Influenza infection is an annual health as well as socio-economic problem in our society. Although the current vaccination method effectively reduces the risk of death, the following issues still remain.

(1) What are the human host factors that contribute to pathogenesis during the acute phase of infection?
It is widely suspected that the avian H5N1 Influenza virus will become a future pandemic virus strain. The lack of an animal model hampers better understanding of how this deadly virus causes severe pathogenic infectious disease in human, especially in terms of the immune system. Using humanized mice, we investigated what factors impact the severe pathogenesis caused by avian H5N1 viral infection. Our gene expression profiling studies demonstrated that type I interferon-related genes are upregulated in human-derived cells in humanized mice by H5N1 viral infection. We are now investigating what human host factors impact on the severe pathogenesis unique to the H5N1 virus.

(2) Generating a next-generation vaccine against a broad range of influenza viruses

Several groups have succeeded in isolating human and rodent antibodies that are broadly reactive with various influenza virus strains including H1N1 and H5N1, findings that point to the feasibility of developing a universal vaccine. These antibodies mostly recognize the stem region of the Hemagglutinin (HA) protein, a region that is well-conserved at the amino acid sequence level. We are designing synthetic peptides to induce B cell antibody responses that can broadly neutralize influenza virus infections. For the first step, using crystal structural data from the complex of HA antigen and broadly-reactive antibody, we performed *in silico* screening of HA peptides able to bind the broadly-reactive antibody. Among them, we identified several peptides that can bind to the broadly-reactive antibodies in biochemical assays. For the next step, we will further modify and test their neutralization activity on various influenza virus strains.

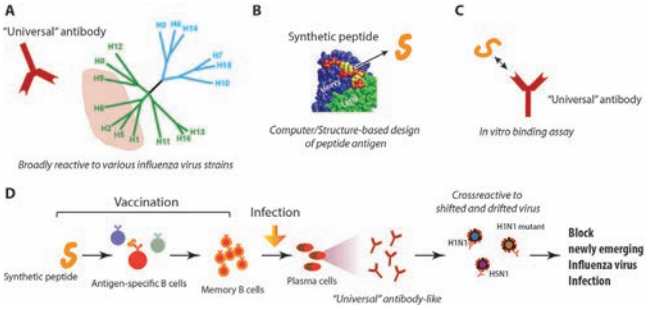


Figure: Crafting universal vaccine for pandemic Influenza
A) Cross-reactivity of a "Universal" antibody. B) Numerous influenza peptides are designed and their affinities for the "universal" antibody are predicted *in silico*. C) Selected peptides are subjected to biochemical binding analyses using the universal antibody. D) Potential peptides for the vaccine will be evaluated for their ability to generate "universal" antibody *in vivo* and to protect animals from infection with various influenza virus strains.

PGRN-CGM International Collaborative Studies

The U.S. NIH Pharmacogenomics Research Network (PGRN) is a consortium of research groups funded as individual cooperative agreements by the NIH. PGRN investigators are top researchers from U.S. academic institutions and conduct studies of variation in human genes relevant to drug metabolism, pharmacokinetics and pharmacodynamics, and the relationship of these genetic variations to drug responses. Principal investigators of the PGRN and RIKEN Center for Genomic Medicine (now RIKEN IMS Core for Genomic Medicine: CGM) held a series of discussions on the need to accelerate discoveries in pharmacogenomics (PGx) and launched the Global Alliance of Pharmacogenetics (GAP) in 2008.

In this international collaboration, the PGRN has been successfully assembling very large collection of DNA samples from well-phenotyped patients receiving specific drugs and drug combinations in clinical trials conducted in the U.S. The CGM focuses on high-throughput genome-wide SNP scans with technological and methodological expertise to identify genetic factors associated with drug responses, risk of severe adverse drug reactions and non-response to medications. Moreover, in 2014, the CGM began a program to discover rare variants by targeted sequencing of selected genes or regions using next generation sequencing (NGS), an approach considered to be complementary to genome-wide association studies (GWAS). Together, the PGRN-CGM capitalizes on these strengths to advance discoveries in PGx research. More than 30 collaborative studies for various drug responses are ongoing to identify genomic biomarkers, which will develop better and safer medications and realize the dream of global personalized medicine.

Figure: Pharmacogenomics Research Network (PGRN) - RIKEN IMS Core for Genomic Medicine (CGM) strategic alliance.
Please visit http://bts.ucsf.edu/pgrn-cgm/

Collaboration with Asian Institutes and SEAPharm

One of the aims of the Laboratory of International Alliance on Genomic Research is to promote research collaborations around the globe. We have established connections with the Institute of Biomedical Sciences at Academia Sinica in Taiwan, where we will explore several diseases and pharmacogenetic studies (PGx) together. In addition, we will also work closely with the Taiwan Biobank to study complex diseases and PGx across the two populations. It has been noticed that rare skin adverse reactions called SJS/TENS occur at a much higher frequency in East Asian and Southeast Asian populations and that these drug-induced adverse reactions have strong genetic associations. To tackle this problem, we established the South East Asian Pharmacogenomics Research Network (SEAPharm) together with six Asian countries (Japan, Korea, Indonesia, Malaysia, Singapore, Taiwan, and Thailand). The aim of the collaborative effort is to identify significant PGx events important to the region so that we can identify genetic markers associated with these adverse drug reactions, which could lead to a reduction in these events. We are now focusing on the identification of genetic associations in phenytoin induced SJS/TEN and anti-TB induced liver injury. In addition, we also aim to understand how the identified genetic markers lead to the adverse events. It is hoped that the discoveries from our collaborative efforts will identify useful biomarkers that can be used to predict drug-induced adverse events, guide drug use and aid in disease prediction/diagnosis.

Figure: South East Asian Pharmacogenomics Research Network (SEAPharm)

International Cancer Genome Consortium (ICGC)

Laboratory for Genome Sequencing Analysis Laboratory for Digestive Diseases Laboratory for Medical Science Mathematics

The ICGC has been organized to launch and coordinate a large number of research projects that have the common aim of comprehensively elucidating the genomic changes present in many types of cancers. Its primary goals are to generate comprehensive catalogues of genomic abnormalities in different cancer types and to make the data available to the entire research community with minimal restrictions. At the end of 2014, 74 cancer genome projects across 16 countries and the EU were ongoing, and the ICGC released the genomic data from 12,232 cancer samples as Release 17 (October, 2014). The RIKEN group has been involved with liver cancer, which is one of the most common and deadly cancers worldwide, especially in Asia. We performed whole genome sequencing (WGS) and RNA-Seq for 270 liver cancers and called their somatic mutations by using our in-house pipeline. We deposited WGS data of all 270 liver cancers and released them as a Japanese ICGC project in Release 18. Combined with data from the National Cancer Research Center (234 samples), the Japanese ICCG project successfully completed the obligated number (500) of cancer genomes. As an internal working group, we are involved with benchmark comparison studies, where eleven genome centers analyze the same raw sequence data or the same DNA by each of their pipelines or platforms to compare their results in somatic mutation identification (Manuscripts submitted). ICGC has launched a "pan-cancer" whole genome project (PCAWG), where ~3000 cancer WGS data will be analyzed in the same pipeline within the same computational environment; 600 researchers and technical staff are involved world-wide in 16 theme working groups (Fig). We are contributing to this ambitious project as a member of a technical working group arranging six "cloud" data centers worldwide, PI and researchers for driver gene analysis, mutational signature, immunogenomics, and mitochondrial genomics, as well as by providing 300 WGS data to the PCAWG (10% contribution 300/3000).

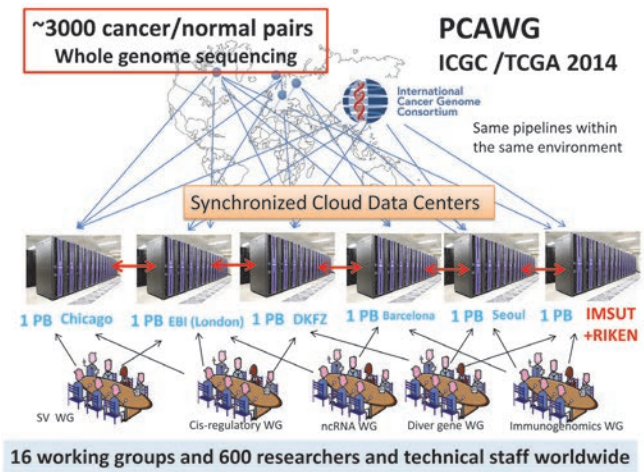


Figure: Overview of the PCAWG ("Pan-CAncer" Whole Genome project) in ICGC/The Cancer Genome Atlas (TCGA).
The ICGC is organizing six synchronized cloud data centers worldwide, each with 1 petabyte (PB) storage capacity, where 2000-3000 WGS pair datasets will be analyzed in the same computational environment. The centers are located in Chicago, USA; The European Bioinformatics Institute (EBI) located on the Wellcome Trust Genome Campus in London, UK; The German Cancer Research Center (Deutsches Krebsforschungszentrum, DKFZ; Barcelona Super Computer Center, Spain; Seoul Electronics and Telecommunications Research Institute, Korea; and, in Japan, The Institute of Medical Science, The University of Tokyo (IMSUT) and RIKEN, IMS. There are 16 working groups (WG) including those analyzing genomic structural variation (SV), *cis*-regulatory elements, non-coding RNA, driver gene mutations, and immunogenomics.

The BioBank Japan Project

Website: http://www.biobankjp.org/english/index.html

Photo: DNA Storage (top) and blood serum storage (bottom)

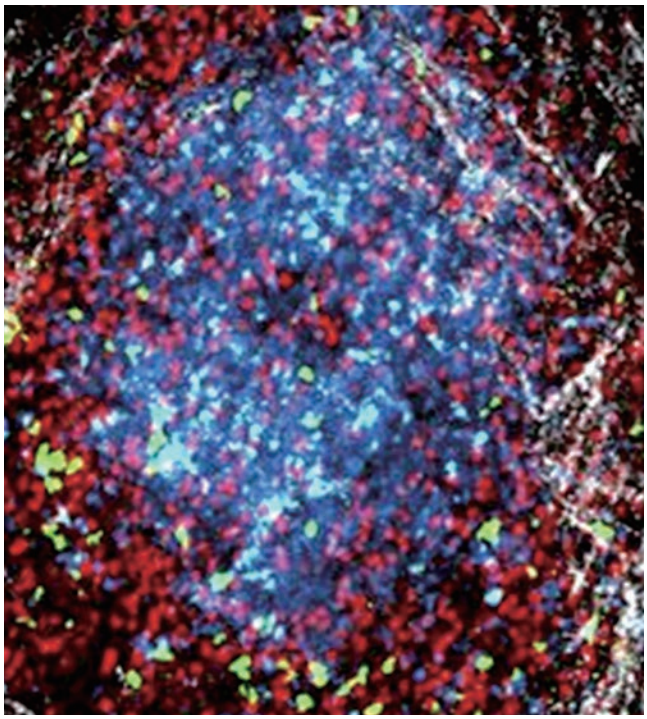
The BioBank Japan project was started as a leading project of the Ministry of Education, Culture, Sports, Science and Technology (MEXT) in 2003 [Project leader: Yusuke Nakamura (FY2003–2011), Michiaki Kubo (FY2011-present)]. In the first 5-year period (FY2003–2007), the project constructed the BioBank Japan, which collected DNA, serum and clinical information from 200,000 patients who suffered from at least one of the 47 target diseases. In the second 5-year period (FY2008–2012), the project performed GWAS for various diseases using the samples stored in the BioBank Japan and identified many susceptibility genes for various diseases and drug responses. In the third 5-year period beginning in 2013, the project is expanding the BioBank infrastructure to collect DNA and clinical information from an independent cohort of 100,000 patients who suffer from at least one of 38 target diseases, including kidney cancer, dementia and depression as new disease targets. In addition, collaboration with 3 National Centers (National Cancer Center, National Center of Neurology and Psychiatry and National Center for Child Health and Development) and 3 clinical research group (National Hospital Organization, Japan Clinical Oncology Group and Japan Children's Cancer Group) was started in 2014. The project is working on the biobanking of samples (DNA, plasma and tissue) collected by clinical research group and the genomic research using the samples stored in National Centers and clinical research groups. To standardize the method of tissue sampling, the project is collaborating with the Japan Society of Pathology to establish the standardized method of tissue sampling for genomic research. In conjunction with this collaboration, the Biobank Japan is expanding its capacity of DNA bank and serum/plasma bank and establishing automated tissue bank. The project completed GWAS of almost all samples collected at the first 5-year period and started whole genome sequencing of 1,000 patients as a pilot project.

Genome-guided drug Therapy Optimization Project (G-TOP)

Website: http://www.biobankjp.org/pgx/index.html

Figure: Website of the Genome-guided drug Therapy Optimization Project (G-TOP)

The Genome-guided drug Therapy Optimization Project was started in December, 2011 as a top-down project of MEXT to validate the clinical utility, clinical efficacy and cost-effectiveness of pharmacogenomic research findings by a clinical intervention study and to implement pharmacogenomic testing into clinical practice [Project leader: Michiaki Kubo (FY2011–2014)]. This project conducted three clinical interventional studies for the prevention of Carbamazepine-induced skin rush (GENCAT), genome-guided dose adjustment of Warfarin for safer anticoagulation (GENCAT), and genome-guided dose adjustment of tamoxifen for breast cancer therapy (TARGET-1). The Core for Genomic Medicine and the BioBank Japan organize all three projects in collaboration with many universities and hospitals in Japan. The Research Group for Pharmacogenomics (Group Director: Taisei Mushiroda) also manages this project. GENCAT and GENWAT study completed the enrollment of study participants in 2014. Final analysis to validate the clinical efficacy of pharmacogenomics testing is ongoing.



Part 3

Events

RIKEN IMS Summer Program (RISP) 2014

IMS was delighted to successfully organize the 9th RISP (RIKEN IMS Summer Program). RISP began as the RCAI International Summer Program (RISP) in 2006 and has now been continued by IMS, beginning last year. The aim of this activity is to provide networking opportunities on a broad international scale for young immunologists as well as to encourage future collaboration and postdoctoral training experiences in Japan. Due to the broadened research activities of the new IMS, the RISP 2014 program was modified somewhat to include topics in genomic study to understand human diseases. The internship program in which the participants perform research in the laboratories of IMS has been maintained. RISP 2014 was co-organized by the Chiba University Leading Graduate School Program.

In RISP 2014, forty-three graduate students and postdoctoral fellows from twelve countries, together with ten students from Chiba University, gathered at Yokohama from June 20th to 27th. The program started with a tour of IMS research facilities, which include advanced two-photon microscopes, a HILO microscope for single molecule imaging and conventional cell sorters as well as a new mass spectrometry-based CyTOF cell sorting instrument. The scientific sessions included 13 lectures by distinguished senior scientists. RISP students also presented their research in both oral and poster sessions. The RISP program ended with participation in a two day International Symposium on Immunology, co-organized by IMS and the Japanese Society for Immunology.

RISP2014 was a success; we received feedback comments on an evaluation survey and all students indicated that they would recommend this program to colleagues. From the other perspective, many lecturers commented about how impressed they were with the quality of RISP students. IMS will again organize RISP in 2015, updating some of the lecture topics as necessary to keep pace with recent developments in this rapidly moving life science field.

The IMS-JSI International Symposium on Immunology 2014

The IMS-JSI International Symposium on Immunology, hosted by the RIKEN Center for Integrative Medical Sciences (IMS), in conjunction with the Japanese Society for Immunology (JSI), was held on June 26-27 at the Pacifico Yokohama conference center. The symposium entitled "Decoding Immune Complexity-Bench to Bedside-" included 18 internationally-recognized speakers presenting their cutting-edged research and attracted close to 400 participants. There were four sessions, the first was "Human genetics and diseases". To date, accumulating datasets from genomic research, such as genome-wide association studies (GWAS), have helped us to some extent in understanding mechanisms of various diseases. However, it is still a big challenge to put all datasets into a comprehensive description of how human diseases arise. This session gave us successful examples of GWAS but also proposed a valuable complementary approach focusing on epigenetic modification. In the "Environment and immune responses" session, unique subsets of, for example, T cells were shown to play distinct roles in immune responses and to affect human diseases. One of the highlights of the "Roadmap to immune cells" session was the high-resolution analysis of hematopoietic cells using single-cell transplantation. Such an approach absolutely brings this research field up to the next level and provided exciting new insights. The "Linking immune and nervous systems" session highlighted a research area studying interactions between nervous and immune systems, a new frontier that will become an even more important field in the coming years. Of note, the symposium this year attracted more individuals from the private sector, such as pharmaceutical companies, and this added value to the event.

The 10th PGRN-RIKEN Strategic Alliance Meeting

The Global Alliance for Pharmacogenomics (GAP), a collaborative program between the former RIKEN Center for Genomic Medicine (now The Core for Genomic Medicine (CGM), RIKEN IMS) and the US National Institute of Health (NIH) Pharmacogenomics Research Network (PGRN), was started in 2008 with the objective of identifying the relationship between genetic variants and individual responses to drugs, including efficacy and side effects. GAP strategic alliance meetings, held alternately in Japan and United States, allow for face-to-face discussions about the progress of ongoing projects and future directions for the PGRN-RIKEN CGM collaboration.

On June 25–26, 2014, CGM hosted the 10th PGRN-RIKEN Strategic Alliance Meeting at the Business Support Floor of the Landmark Tower Yokohama, in Yokohama, Japan. Some 40 participants from PGRN and RIKEN attended this meeting, and it provided a valuable forum for exchanging information on ongoing collaborative activities: "Addition of bevacizumab to standard carboplatin-paclitaxel chemotherapy in patients with advanced ovarian cancer", "Systems Pharmacology: genomic discoveries in a minority population", "Updates on CALGB 80303", "Patients Experiencing Breast Events While Receiving Aromatase Inhibitors for Early Breast Cancer on NCIC CTG Trial MA.27", and "Pathway GWAS: Expanding the universe of asthma pharmacogenetic studies". Participants also explored five new collaborative research proposals from PGRN and finally decided to adopt three as new collaboration projects. In addition, Dr. Yukihide Momozawa from CGM presented a newly developed targeted resequencing method.

PGRN and RIKEN members had in-depth discussions and shared ideas to further enhance collaborations and another successful meeting concluded with plans for the next one.

The 1st IMS Symposium

On February 28, 2014, the RIKEN Center for Integrative Medical Sciences (IMS) held its first public symposium on new medical sciences for the advancement of personalized and preventive medicine at Station Conference Tokyo.

This symposium was attended by 200 participants, including researchers from universities, various research institutes, and pharmaceutical and medical companies.

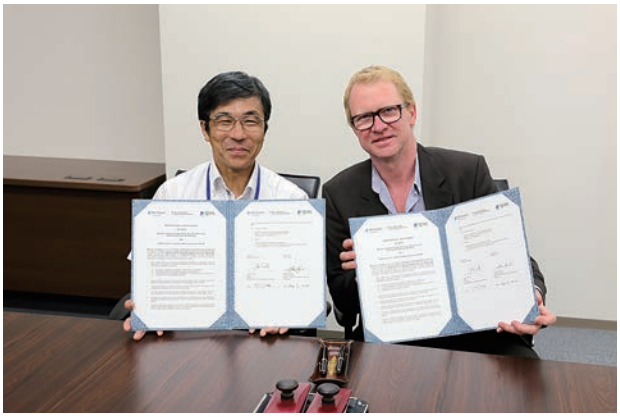
After the opening greetings by Mr. Yutaka Hishiyama, Deputy Director-General of the Office of Healthcare Policy in the Cabinet Secretariat and Mr. Yoshinori Horiuchi, Director of the Life Sciences Division in the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Dr. Shigeo Koyasu, IMS Director (Acting Director at the time) introduced the IMS targets and strategies. Seven principal investigators from the center's different research sections spoke about their frontline research activities to advance personalized and preventive medicine in terms of predicting disease in the individual and the development of preventive methods and treatments tailored to the individual.

The keynote lectures were delivered by Dr. Masato Kasuga, President of the National Center for Global Health and Medicine and Dr. Kazuhiro Sakurada, Senior Researcher at Sony Computer Science Laboratories. Dr. Kasuga spoke about pathological diabetes from a homeostatic perspective, while Dr. Sakurada described a new systems medicine approach for proactive medical treatment.

RIKEN IMS-Monash University Collaborative Agreement

International collaboration plays a key role in the field of medical sciences. IMS has already concluded many collaboration agreements with research institutions worldwide but, so far, these have mostly been in Europe and the US. IMS had an opportunity to interact with Monash University in Melbourne, Australia in 2013. Monash University is very active in regenerative medicine, immunology and systems biology and is well-known as the host of EMBL Australia at the Australian Regenerative Medicine Institute. Their research direction nicely fits with IMS and thus collaboration with them can complement research activities on both sides and eventually improve the international visibility of IMS. More importantly, Monash University is also keen to connect Japanese and Australian research and industry partners through various research collaborations. In this context, IMS held a one-day symposium at the RIKEN Yokohama campus on August 5th, 2014, with delegates from Monash University to exchange ideas for future collaborations. Taking this opportunity, IMS concluded a Memorandum of Understanding with Monash University through its Faculty of Biomedical and Psychological Sciences for enhancement of research interactions. Bottom-up collaborations have already started between some individual laboratories. IMS and Monash University will continue to pursue more opportunities to enhance each others research activities through this collaboration.

Photo: Conclusion of a Memorandum of Understanding with Monash University.
Dr. Shigeo Koyasu, IMS Director (left) and Professor John Carroll, Dean and Head of Biomedical and Sciences, Monash University (right) at the signing ceremony.



RIKEN IMS-LCSB Cooperative Agreement

On October 10th, 2014, IMS and the University of Luxembourg acting for its Luxembourg Centre for Systems Biomedicine (LCSB) agreed to a cooperative research program to understand the role of the immune system in health and diseases. The agreement was signed during the visit of Luxembourg's economic delegation to Japan, led by Luxembourg's Crown Prince Guillaume.

Prior to the signing ceremony, a life science workshop was held at the Luxembourg Embassy in Japan. Mr. Mario Grotz, Director General for Research, Luxembourg's Ministry of Economy, Dr. Haruhiko Koseki, Deputy Director of IMS, Dr. Hiroaki Kitano, President of the Systems Biology Institute (SBI) and Group Director of IMS, and Professor Rudi Balling, Director of LCSB discussed their interdisciplinary research approaches and Drs. Koseki and Kitano introduced two ongoing collaborative research projects between LCB and RIKEN or SBI.

After the seminars, Dr. Shigeo Koyasu, Director of IMS, and Professor Balling signed the partnership agreement at Academy Hills Club in central Tokyo. The agreement includes to 1) combine the Centers' strengths in systems and experimental biology and clinical expertise to improve the understanding of molecular and cellular mechanisms underlying disease pathogenesis and eventually develop personalized medicine approaches and 2) seek funding for their activities through respective institutional funds or funding agencies for research programs of Luxembourg and Japan.

The day concluded with an official reception in the presence of the Luxembourg's royal couple Prince Guillaume and his wife Princess Stéphanie, Mr. Étienne Schneider, Deputy Prime Minister and Minister of the Economy and Mr. François Bausch, Minister for Sustainable Development and Infrastructure.

Photo: (From right to left) Mr. Étienne Schneider (Luxembourg's Deputy Prime Minister and Minister of Economy), Professor Rudi Balling (Director, LCSB), Dr. Shigeo Koyasu (Director, RIKEN IMS) and Mr. François Bausch (Luxembourg's Minister for Sustainable Development and Infrastructure)

France-Japan Immunology Meeting

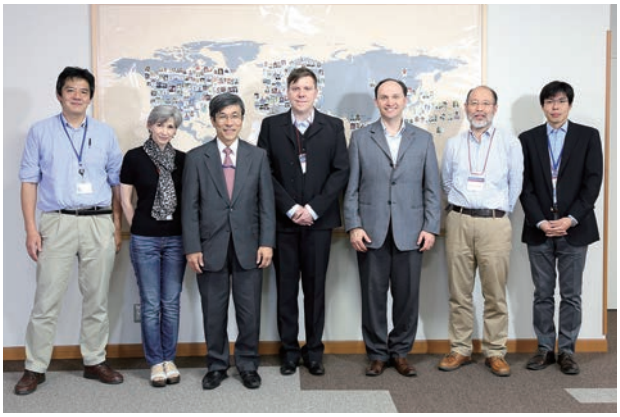
Joint workshops on Immunology between Pasture Institute/INSERM and the former RIKEN RCAI have been organized several times in the past. The most recent workshop was held on October 22–23 in Cassis, a beautiful city in Provence, France. This time, there was a broader range of participating institutions and research on both sides of the Immunology workshop; France included the Pasture Institute, CIML Marseille and INSERM and Japan had RIKEN-IMS and IFReC Osaka University.

The meeting started with a few words of welcome by the city mayor. The workshop highlighted a wide range of immunology research, from basic to clinical applications. There were four sessions on the first day: Host-pathogen Interactions, Regulation of Mucosal Immunity, Cancer, and Autoimmunity and Tolerance, and three sessions on the second day: Lymphocyte Function and Dynamics, Inflammation and Immune System, and Immune Regulation by DC and Myeloid Cells. Thirteen French and twelve Japanese speakers (6 from RIKEN-IMS, 6 from IFReC) presented cutting edge research in these sessions and discussed/exchanged critical information. At the close of the meeting, both sides agreed on the importance of having an exchange of young researchers between the participating laboratories, as well as continuing the joint workshop.

RIKEN-McGill Mini-Workshop

The RIKEN-McGill Mini-workshop was held on May 12-13, 2014 at IMS. RIKEN and McGill University, one of Canada's best-known and leading universities, signed an agreement in 2010 to cooperate for staff exchange, collaborative research activities and joint academic meetings. Under this framework, a series of joint workshops have been held focusing on green chemistry and nanosciences in 2010 and 2012, and biomedical fields in 2013. At the biomedical joint workshop in 2013, Drs. Piccirillo, Fritz and King from the Department of Microbiology and Immunology in McGill University and Drs. Koyasu, Koseki and Hori from RIKEN IMS agreed to initiate further discussion of collaborative research toward a better understanding of homeostatic mechanisms underlying host/pathogen interactions.

In May 2014, with the support by RIKEN-McGill collaboration funding, Drs. Piccirillo and Fritz visited IMS to attend the RIKEN-McGill joint workshop. On the first day, there were presentations by IMS and McGill researchers. Dr. Fritz introduced his research on the regulation of mucosal inflammation, and Drs. Fagarasan, Ohno and Honda talked about bacteria-immune symbiosis in the gut and induction of colonic Treg or Th17 cells. Then, Dr. Piccirillo discussed the functional dynamics of Treg in human. On the second day, Drs. Piccirillo and Fritz had one-on-one discussions with IMS researchers on potential topics for collaboration. After the meeting, researchers could find many common interests and agreed to facilitate the synergy between IMS and McGill University to solidify these prospective research themes.



RIKEN IMS Advisory Council 2014

The inaugural IMS Advisory Council was held on May 28th and 29th, 2014 at RIKEN IMS in Yokohama. The Advisory Council members were Drs. Max Cooper (Chair), Mark Lathrop (Vice Chair), Hiroyuki Aburatani, Rudi Balling, Michael Georges, Ronald Germain, Hajime Karasuyama, Yutaka Kawakami, Paul Kincade, Edison Tak-Bun Liu, Bernard Malissen, John O'Shea, William Paul, Fiona Powrie, Peter Sorger, Kiyoshi Takatsu, Katsushi Tokunaga, Dale Umetsu and Arthur Weiss. On the first day there were block reviews, where Advisory Council members reviewed individual laboratories in their field of expertise. The second day was the Assembly Meeting, where there were presentations outlining the Center's Future Plans.

At the end of the two-day meeting, the Advisory Council summarized their findings and reported to Dr. Shigeo Koyasu (IMS Director, Acting) and Dr. Maki Kawai (RIKEN Executive Director). They commented that IMS was a logical joining of two great institutes and that the merger created the capacity to perform functional genomics, which could set IMS apart from other genomics institutes in the world. Dr. Cooper, the chair of the Advisory Council, commented that there were outstanding investigators present in the new institute and there had already been some integrated planning, thus the potential was there that this new institute would succeed. In terms of moving the IMS center forward during this transition period, the Advisory Council pointed out an urgent need of a permanent Director and, at least, a stable level of funding to help foster the success of the new center.

IMS Retreats

IMS held both its first and second Retreat in 2014. The aim of these retreats was to give IMS researchers a better understanding of each other's research focuses and techniques, because IMS only started in 2013 as an ensemble of groups with heterogeneous research backgrounds.

The first retreat was held at Shonan Village Center on February 3rd and 4th, 2014. The 183 researchers and students listened to talks from Dr. Koyasu and Dr. Kubo about the new Center's strategies, and then each lab presented at least one poster to introduce the laboratory's research focus and strategies. On the second day, nine selected young researchers gave oral presentations.

For the second IMS Retreat, 191 IMS members gathered at Narita View Hotel on Oct. 9th and 10th, 2014. Dr. Rudi Balling, a member of the IMS Advisory Council, joined as a guest speaker. On the first day, following Drs. Koyasu, Kubo, Balling and Ohara's talks, young researchers or technical staff each had one minute to introduce their research focus. During dinner, there was a surprise announcement from RIKEN that Dr. Koyasu had been appointed as the Director of IMS.

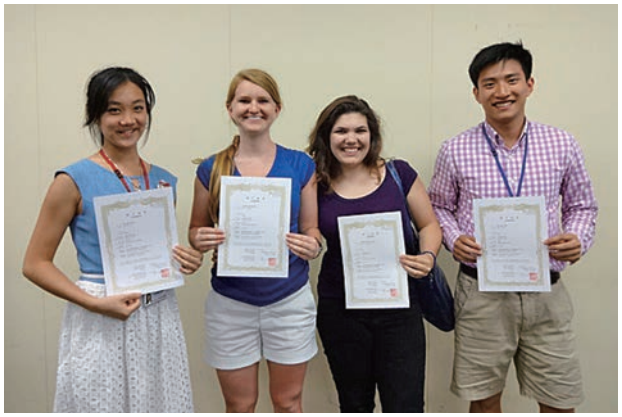
Thanks to the organizers and the working group members of the 1st retreat (Takaharu Okada, Akihiro Fujimoto, Hisahiro Yoshida, Rieko Okoshi, Kozue Fujisawa, Mayumi Mirokuji, Akiko Imaizumi, Yosuke Nakayama, Hiroko Yamaguchi, Haruka Iwano) and the 2nd retreat (Tatsuhiko Tsunoda, Shin-ichiro Fujii, Takaharu Okada, Kozue Fujisawa, Mayumi Mirokuji, Aiko Iyama, Akiko Imaizumi, Yosuke Nakayama, Kie Sunatori), the two retreats provided excellent opportunities for researchers to communicate with each other, especially for the young scientists and students.

Harvard Summer School 2014

IMS offers a summer internship program for undergraduate students from Harvard University. In this program students do a research internship in the IMS laboratories, have basic biomedical sciences lectures by IMS PIs and attend a Japanese language course. They also participate in the RIKEN IMS Summer Program (RISP) and the RIKEN IMS-JSI International Symposium on Immunology. The participants receive a grade from IMS and course credit from Harvard. In 2014 from June 2 to August 11, there were three students from Harvard University (Elaine Dong, Karen L. Kennedy, and L. Mica Yoder), and one from Yale University (George Mo).

Ms. Dong conducted her research project in the Laboratory for Inflammatory Regulation (Dr. Tanaka), Ms. Kennedy in the Laboratory for Disease Systems Modeling (Dr. Kitano), Ms. Yoder in the Laboratory for Human Disease Models (Dr. Ishikawa), and Mr. Mo in the Laboratory for Vaccine Design (Dr. Ishii). During their internships, the students had numerous discussions with IMS researchers and, at the end of the program, they gave oral presentations describing their research results.

In addition, they visited the Science Frontier High School and met with students who want to be scientists. On this occasion, they experienced Japanese culture and learned Sado (tea ceremony) from the local high school students.



Adjunct Professorship Programs

IMS collaborates with and accepts graduate students from 10 domestic university graduate schools. There are now a total of 35 adjunct professors/associate professors in IMS (Table), and 119 students studied at IMS in 2014. On Apr 26, IMS held a briefing session on adjunct graduate school programs. Sixteen students participated from Hokkaido, Miyagi, Tokyo, Saitama, and Kanagawa prefectures. The session provided an opportunity for students to visit and talk directly with lab leaders and to consider their future directions.

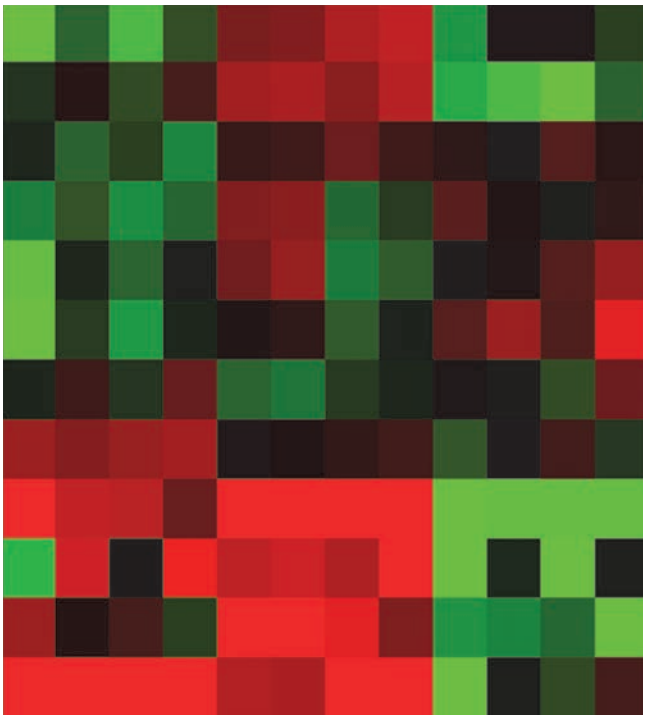
Table: Joint graduate school programs

Graduate Program	Affiliated IMS Investigator
Graduate School of Frontier Biosciences, Osaka University	Tomohiro Kurosaki (Professor), Ichiro Taniuchi (Visiting Professor)
Graduate School of Medicine, Osaka University	Takashi Saito (Visiting Professor), Takashi Tanaka (Visiting Professor)
Graduate School of Medicine, Chiba University	Takashi Saito (Visiting Professor), Haruhiko Koseki (Visiting Professor), Hiroshi Ohno (Visiting Professor), Shin-ichiro Fujii (Visiting Associate Professor), Yasuyuki Ishii (Visiting Associate Professor), Fumihiko Ishikawa (Visiting Associate Professor)
Graduate School of Pharmaceutical Sciences, Chiba University	Osamu Ohara (Visiting Professor)
Graduate School of Biomedical Science, Tokyo Medical and Dental University	Takashi Saito (Visiting Professor)
Graduate School of Medicine, Yokohama City University	Michiaki Kubo (Visiting Professor), Shiro Ikegawa (Visiting Professor), Mayumi Tamari (Visiting Professor), Tatsuhiko Tsunoda (Visiting Professor), Hidewaki Nakagawa (Visiting Professor), Taisei Mushiroda (Visiting Professor), Atsushi Takahashi (Visiting Professor)
Graduate School of Medical Life Science, Yokohama City University	Hiroshi Ohno (Visiting Professor), Makoto Arita (Visiting Professor), Mariko Okada (Visiting Professor), Takaharu Okada (Visiting Associate Professor), Kazuyo Moro (Visiting Associate Professor)
Research Institute of Biological Sciences, Tokyo University of Science	Masato Kubo (Professor), Osamu Ohara (Visiting Professor), Shohei Hori (Visiting Associate Professor), Tadashi Yokosuka (Visiting Associate Professor)
Graduate School of Medicine, Kyoto University	Fumihiko Ishikawa (Visiting Associate Professor)
Graduate School of Medicine, Keio University	Masayuki Amagai (Professor), Kenya Honda (Professor), Shigeo Koyasu (Visiting Professor), Haruhiko Koseki (Visiting Professor)

Guest Lectures 2014

Table: Guest Lectures Jan–Dec, 2014

Date	Speaker	Affiliation	Country	Title
16-Jan-14	Dr. Laurent Renia	Singapore Immunology Network-BMSI-A*STAR	Singapore	Cerebral malaria: immunological mysteries at the blood brain barrier
30-Jan-14	Dr. Ruth Nussinov	Cancer and Inflammation Program, National Cancer Institute and Tel Aviv University	Israel	How can computational structural biology help cancer research?
27-Jan-14	Dr. Maria Curotto de Lafaille	Singapore Immunology Network-BMSI-A*STAR	Singapore	Regulation of IgE cell differentiation and memory
26-Feb-14	Dr. Junzo Takeda	National Hospital Organization, Tokyo Medical Center	Japan	Anaphilaxis induced by muscle relaxant and cyclodextrin (in Japanese)
12-Mar-14	Dr. James Douglas Engel	Department of Cell and Developmental Biology, University of Michigan Medical School	USA	GATA transcription factor regulation of HSC homeostasis and T cell development
20-Mar-14	Dr. David Schlessinger	Laboratory of Genetics, Intramural Research Program, National Institute on Aging, National Institutes of Health	USA	Genetics and aging
24-Apr-14	Dr. Naoyuki Kamatani	StaGen Co., Ltd.	Japan	Can Drug Discovery in Japan Escape the Valley of Death? —Successful Examples of Genome-Based Drug Discovery (in Japanese)
24-Apr-14	Dr. Cameron Osborne	Department of Medical and Molecular Genetics, King's College London School of Medicine	UK	High-resolution capture Hi-C to map long-range promoter contacts in human cells
28-Apr-14	Dr. Eiji Hara	Division of Cancer Biology, The Cancer Institute, Japanese Foundation for Cancer Research	Japan	Cellular senescence and cancer
3-Jun-14	Dr. Masaki Miyazaki	Division of Biological Sciences, University of California San Diego	USA	The balance between the transcription factors E-protein and its agonist Id-protein that orchestrates naive T cell fate and maintains regulatory T cell function
19-Jun-14	Dr. Huiying Li	Department of Molecular and Medical Pharmacology, University of California Los Angeles	USA	The human skin microbiome in acne
15-Aug-14	Dr. Tetsuo Shiohara	Department of Dermatology, Kyorin University School of Medicine	Japan	Drug eruption: current status and the future challenges (in Japanese)
25-Sep-14	Dr. Pierre-Antoine Defossez	Epigenetics and Cell Fate, Le Centre National de la Recherche Scientifique (CNRS)	France	Biological functions of mammalian methyl-binding proteins
20-Oct-14	Dr. Yasutaka Okabe	Department of Immunobiology, Yale University School of Medicine	USA	Functional specialization of tissue-resident macrophages
23-Oct-14	Dr. Aktar Ali	Department of Internal Medicine, University of Texas Southwestern Medical Center	USA	SNS-032: Targeting tumor angiogenesis, invasion and metabolism
29-Oct-14	Dr. Hiroshi Haeno	Department of Biology, Kyushu University	Japan	Mathematical modeling of cancer progression
17-Nov-14	Dr. Tamotsu Yoshimori	Department of Genetics, Osaka University Graduate School of Medicine	Japan	Autophagy: molecular mechanisms and pathophysiological basis (in Japanese)
25-Nov-14	Dr. James J. Lee	Department of Biochemistry and Molecular Biology, Mayo Clinic	USA	Pulmonary responses following allergen provocation in mice result from the differential activation of airway eosinophils promoting the expression of IL-13
25-Nov-14	Prof. Antoine H.F.M. Peters	Friedrich Miescher Institute for Biomedical Research (FMI)	Switzerland	Epigenetic control of mammalian germ line and early embryonic development
26-Nov-14	Prof. Atsushi Shimizu & Associate Prof. Tsuyoshi Hachiya	Division of Biomedical Information Analysis, Iwate Tohoku Medical Megabank Organization	Japan	Technology development for genome-based disease prediction (in Japanese)
2-Dec-14	Dr. Yohei Hayashi	Cardiovascular Disease, Gladstone Institute	USA	Studies of patient-specific induced pluripotent stem cells: examples from ring-chromosomal patients
4-Dec-14	Dr. Satoshi Namekawa	University of Cincinnati/ Cincinnati Children's Hospital Medical Center	USA	Polycomb functions in germline epigenome and sex chromosome inactivation
16-Dec-14	Dr. Takeshi Egawa	Department of Pathology and Immunology, Washington University School of Medicine	USA	Transcriptional regulation of durable $CD8^+$ T cell responses against microbial infection



Part 4

Data and Statistics

Publlications 2014

Table: IMS Publlications Jan-Dec, 2014

Journal	Impact Factor (2013)	Number of Papers
N Engl J Med	54.4	1
Nature	42.4	5
Nat Rev Drug Discov	37.2	1
Cell	33.1	1
Science	31.5	2
Nat Genet	29.6	6
Nat Immunol	25.0	7
Cell Stem Cell	22.2	1
Immunity	19.7	6
Neuron	16.0	1
J Am Coll Cardiol	15.3	1
Circulation	14.9	1
J Exp Med	13.9	2
Gut	13.3	1
Immunol Rev	12.9	1
Cell Host Microbe	12.2	1
J Allergy Clin Immunol	11.2	5
Hepatology	11.2	1
Am J Hum Genet	11.0	1
Nat Commun	10.7	6
Dev Cell	10.4	1
Proc Natl Acad Sci U S A	9.8	10
Blood	9.8	2
Leukemia	9.4	1
Cancer Res	9.3	1
Nucleic Acids Res	8.8	1
Diabetes	8.5	3
EMBO Mol Med	8.2	1
Clin Cancer Res	8.2	1
EMBO Rep	7.9	1
Clin Pharmacol Ther	7.4	3
Cell Rep	7.2	3
Diabetologia	6.9	1
Hum Mol Genet	6.7	6
J Bone Miner Res	6.6	1
Semin Immunopathol	6.5	1
Sci Signal	6.3	1
Am J Transplant	6.2	1
Allergy	6.0	1
Others		135
Total		**226**

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Budget, Personnel and Patents

IMS Budget FY2014

IMS Budget FY2014	JPY Million
Government funding for operations	3,421
Commissioned research	1,586
External competitive funding	1,032
Total	**6,040**

Patents 2014

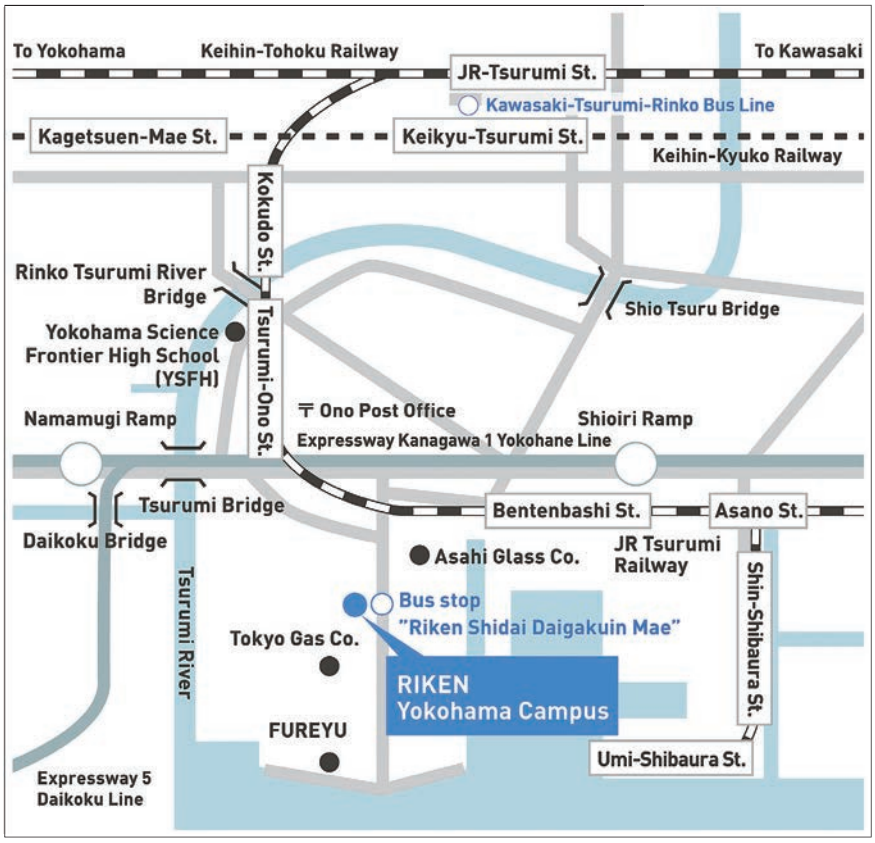
There were 32 patents filed from January to December in 2014.

Patents	Total	International patents (PCT)	Domestic patents (Japan)
2013	32	20	12
2014	32	24	8

Personnel FY2014

Category	Number
Director	1
Senior Advisor	2
Deputy Director	4
Group Director	8
Team Leader	25
Deputy Team Leader	6
Senior Scientist	22
Postdoctoral Researcher	17
Special Postdoctoral Researcher	4
Foreign Postdoctoral Researcher	2
Research Scientist	42
Research Fellow	9
Student Trainee	98
International Program Associate	5
Research Associate	8
Junior Research Associate	16
Technical Staff	126
Coordinator	2
Assistant	32
Part-time Staff	19
Senior Technical Scientist	2
Technical Scientist	7
Research Consultant	2
Consultant	13
Senior Visiting Scientist	8
Visiting Scientist	142
Visiting Technician	32
Visiting Researcher	6
Temporary Staffing	11
Total	**671**

Access to RIKEN Yokohama Campus



Local Access

By Bus
Take the #08 bus from Platform 8 at the East Exit of Tsurumi Station (also accessible from the West Exit of Keikyu Tsurumi Station) and get off at the RIKEN Shidai Daigakuin Mae bus stop. The institute is across the street. All buses from this platform are bound for Fureyu.

Buses depart Tsurumi every 5–15 minutes. It takes about 15 minutes to arrive at RIKEN Yokohama. The fare is 220 yen in cash.

By Train
A 15-minute walk from JR Tsurumi-Ono Station (JR Tsurumi Line), which is directly accessible by transfer from JR Tsurumi Station.

Trains run about every 10 minutes during morning and evening rush hour, but less frequently at other times.

Searchable train timetables in English are available at http://www.hyperdia.com/en/

By Taxi
Use the taxi stand at the East Exit of JR Tsurumi Station or the West Exit of Keikyu Tsurumi Station. The trip takes about 10 minutes and costs around 1,200 yen.

From the Airport

From Haneda Airport

Route 1
Take the Keikyu Railways Airport Express* (blue kanji sign) for Yokohama and get off at Keikyu Tsurumi Station (27–29 minutes). Airport Express trains run every 10-15 minutes between 9:30 a.m. and 9:30 p.m. Next, follow the Local Access directions above to get to RIKEN Yokohama.

Route 2
Take any train marked with a green (express), red or dark grey kanji sign to Keikyu Kamata Station. Transfer to the Keikyu Main Line and take a local train* toward Yokohama until Keikyu Tsurumi Station* (12 minutes).

*Only Airport Express (blue kanji sign) and local trains (dark grey kanji sign) stop at Keikyu Tsurumi Station. Note that Keikyu Tsurumi Station and JR Tsurumi Station are two different railway stations and are separated by a bus rotary (the stations are about 150 meters apart).

From Narita Airport

From Narita Airport Station take the JR Sobu Line (Rapid Express), Airport Limousine Bus or JR Narita Express* to JR Shinagawa Station. (JR Sobu Line is the most inexpensive option and takes about 1 hour and 15 minutes). From JR Shinagawa Station take the JR Keihin Tohoku Line (Yokohama direction) to JR Tsurumi Station (18 minutes). Next, follow the Local Access directions above to get to RIKEN Yokohama.

* A reserved seat express that requires payment of a surcharge in addition to train fare.

Searchable train timetables in English are available at http://www.hyperdia.com/en/

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Annual Report 2014

Cover image: Preparation of glass-supported lipid bilayers for analyzing molecular dynamics of microclusters in Jurkat T cells.
Image courtesy of Laboratory for Molecular Live-cell Quantification.

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